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An activity-based metagenomic screen reveals: RubisCO is not the only enzyme that converts ribulose-1,5-bisphosphate

Stefanie Böhnke-Brandt*, Mirjam Perner
Geomar Helmholtz Centre for Ocean Research Kiel, Germany

Almost all the carbon on earth is assimilated via the Calvin-Benson-Bassham cycle. One of its key enzymes is the ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO). It catalyzes the carboxylation of ribulose-1,5-bisphosphate, forming 3-*D*-phosphoglycerate. RubisCO is the world's most abundant enzyme and can be found in 99.5% of all autotrophic organisms including plants, algae and cyanobacteria but also many autotrophic bacteria and archaea. Given the unculturable majority of microbes, a tremendous potential for novel RubisCOs can be expected in the environment.

To target this black box, we recently developed an activity-based screen which enables us to detect recombinant RubisCO active enzymes from metagenomic libraries. Among 15 000 fosmid clones with hydrothermal deep-sea vent origin, 41 exhibited RubisCO activity. One of these RubisCO active clones was particularly interesting because its DNA insert resembled that of Epsilonproteobacteria (recently reclassified as Epsilonbacteraeota) (61 to 90% AA identity). This group is not known to use the Calvin-Benson-Bassham cycle for autotrophic carbon fixation, but uses the reverse tricarboxylic acid cycle instead. Indeed, none of the identified genes encoded on the fully sequenced insert had homologies to any known RubisCO although this is so far the only enzyme known to convert ribulose-1,5-bisphosphate. Nevertheless, the enzymes expressed from the identified metagenomic fosmid clone converted all of the provided ribulose-1,5-bisphosphate (50mM) in the RubisCO assay within a few seconds. In contrast to a classical RubisCO catalyzed reaction, no 3-*D*-phosphoglycerate is produced. Future work will focus on identifying the gene products responsible for the ribulose-1,5-bisphosphate conversion and on defining the reaction product.

Mainstreaming microbes in global ocean observing: opportunities and challenges for enhanced ecosystem assessment

Pier Luigi Buttigieg*¹, Eduard Fadeev², Christina Bienhold², Laura Hehemann², Pierre Offre³, Antje Boetius²

¹*Alfred-Wegener-Institut Helmholtz-Zentrum für Polar- und Meeresforschung, Germany*, ²*Alfred-Wegener-Institut, Helmholtz-Zentrum für Polar- und Meeresforschung, Germany*, ³*NIOZ Royal Netherlands Institute for Sea Research, Department of Marine Microbiology and Biogeochemistry, Netherlands*

Microbial responses to natural and human-induced environmental variation can serve as multi-layered indicators of ecosystem state and health. Currently, the dynamics of specific populations or functional guilds, including pathogens and toxic algae, or entire communities have been used to further scientific understanding and to advise decision makers on issues of societal concern. Despite its promise, marine

microbial ecology has yet to reach its full potential in long-term, globally coordinated ocean observation. National and regional efforts are often isolated from one another and massive regions of the oceans are undersampled in time and space. As we rapidly approach the United Nations Decade of Ocean Science for Sustainable Development, there is a great need to rally these considerable, but fragmented, capacities towards a coordinated solution which may be integrated into operational systems. We assessed the spatiotemporal coverage of existing long-term marine microbial observatories, revealing regions in clear need of increased attention. While multi-omic approaches emerged as the most feasible route to fill these gaps and produce globally coherent data flows, we noted several key considerations to ensure robustness and sustainability as technologies change. Finally, we propose a path forward, synchronising the marine microbial community with activities such as the development of Essential Ocean Variables by the Global Ocean Observing System, the Essential Biodiversity Variables, and the efforts of the Marine Biodiversity Observation Network. While the challenges in creating global capacities for marine microbial observation are formidable, they are outweighed by the opportunity to mainstream its value in monitoring and managing marine biodiversity.

Microscale turbulent structure determines physiological stress response in motile phytoplankton

Francesco Carrara^{*1}, Anupam Sengupta¹, Lars Behrendt¹, Assaf Vardi², Roman Stocker¹

¹ETH Zurich, Switzerland, ²Weizmann Institute, Israel

Turbulence is a primary determinant of phytoplankton fitness and migration. Microscale turbulence is characterized by Kolmogorov-scale eddies with varying kinematics (eddy rotation rate) and intermittency (interval between two subsequent eddies). Yet, how such properties of turbulent signals impact the behavioral and physiological response of phytoplankton have remained unknown. Here, by combining experiments and modelling, we map the dynamics of stress response on properties of hydrodynamic forcing, and demonstrate its consequences on the phytoplankton migration and physiology. Upon exposure to turbulent cues within a microfluidic chamber, a population of a toxic marine alga *Heterosigma akashiwo* was observed to undergo characteristic split into two sub-populations, each migrating vertically in opposite directions. The population split was modulated by varying the kinematics and intermittency of the turbulent eddies. The split could be triggered upon loading the cells with hydrogen peroxide – a common reactive oxidative species (ROS) – even in the absence of turbulence. The split was however suppressed when cells grown in antioxidant scavenger (potassium iodide) were exposed to the turbulent cues. Our experiments further revealed that the accumulated ROS levels, quantified using flow-cytometry, reduced sharply over the first three minutes after turbulence ceases to act. ROS quantification along with photophysiology experiments showed that the subpopulation migrating upward accumulated lower ROS levels, had higher quantum yield, and showed a faster growth rate relative to the downward migrating cells. These results suggest that the

temporal structure of turbulence – even at rapid time scales – can have long term ramifications on the behavior and physiology of motile phytoplankton.

Marine phototroph-heterotroph interactions propelled by cross-feeding

Joseph A. Christie-Oleza*, Despoina Sousoni, David J. Scanlan
University of Warwick, United Kingdom

Biological interactions underpin the functioning of marine ecosystems, be it via competition, predation, mutualism, or symbiosis processes. Microbial phototroph-heterotroph interactions propel the engine that results in the biogeochemical cycling of individual elements and are critical for understanding and modelling global ocean processes. Here, we performed long-term phototroph-heterotroph co-culture experiments under nutrient-amended and natural oligotrophic seawater conditions which showed that it is not the concentration of nutrients but rather their circulation that maintains a stable interaction and a dynamic system. Using a broad range of model phototrophic microorganisms (i.e. picocyanobacteria *Prochlorococcus* and *Synechococcus*, green algae *Micromonas* and *Ostreococcus*, the haptophyte *Emiliania huxleyi*, and diatoms *Phaeodactylum tricornutum* and *Thalassiosira pseudonana*) grown in combination with a diverse range of twelve marine bacteria we were able to determine interactions that supported long-term growth. The high-throughput proteomic data generated from these co-cultures revealed mechanistic insights of the cross-feeding process that occurs in each one of these systems. For the first time, we present a comprehensive understanding of the networks of marine phototroph-heterotroph interactions at a broad scale and challenge the general belief that marine phototrophs and heterotrophs compete for the same scarce nutrients and niche space, but instead suggest these organisms more likely benefit from each other because of their different levels of specialization and complementarity within long-term stable-state systems.

Single-amino acid variants point to adaptive processes as the main driver of the evolution of a single SAR11 population

A. Murat Eren^{*1}, Tom O Delmont¹, Evan Kiefl¹, Ozsel Kilinc², Ozcan C Esen¹, Ismail Uysal², Michael S Rappé³, Stephen J Giovannoni⁴
¹*University of Chicago, United States*, ²*University of South Florida Tampa, United States*, ³*University of Hawaii at Manoa, United States*, ⁴*Oregon State University, United States*

The diversity and geographical distribution of populations within major marine microbial lineages are largely governed by temperature and its co-variables. However, neither the mechanisms by which genomic heterogeneity emerges within a single population nor how it drives the partitioning of ecological niches are well understood. Here we took advantage of billions of metagenomic reads to study one of the most abundant and widespread microbial populations in the surface ocean.

We characterized its substantial amount of genomic heterogeneity using single-amino acid variants (SAAVs), and identified systematic purifying selection and adaptive mechanisms governing non-synonymous variation within this population. Our Deep Learning analysis of SAAVs across metagenomes revealed two main ecological niches that reflect large-scale oceanic current temperatures, as well as six proteotypes delineating more subtle niche demarcations. We identified significantly more protein variants in cold currents and an increased number of protein sweeps in warm currents, exposing a global pattern of alternating genomic diversity for this SAR11 population as it drifts along with surface ocean currents. Overall, the geographic partitioning of SAAVs suggests adaptive processes, rather than neutral evolution, is the main driver of the evolution of SAR11 in surface oceans.

Coral Reef Microbiomes: Establishing baselines for microbial based monitoring

Bettina Glasl^{*1}, David Bourne¹, Nicole Webster²

¹*James Cook University, Australia*, ²*Australian Institute of Marine Science, Australia*

Coral reefs are becoming increasingly threatened by local and global pressures. The development of effective monitoring tools is therefore of utmost importance for the protection and persistence of coral reefs. Although the fundamental contribution of microbes to the stability and functioning of coral reefs is widely recognised, their potential value for ecosystem diagnostics remains unexplored. An absence of microbial baselines for coral reefs has hindered our ability to detect shifts in the microbial community that could be informative of environmental anomalies. This study established the first comprehensive microbial reference dataset for selected inshore Great Barrier Reef sites and assessed their potential utility in environmental monitoring. A microbial census, including multiple coral reef niches (i.e. seawater, sediment, corals, sponges and macroalgae) assessed at high temporal resolution, was acquired by sequencing the 16S rRNA gene of 381 samples over the course of 16 months. Furthermore, temporal, spatial and intra-niche stabilities of microbiomes with varying lifestyles (e.g. free-living, host-tissue associated and host-biofilm associated) were compared to select optimal microbial traits for microbial monitoring approaches. Fine-scale effects of environmental fluctuations on the compositional variability/stability of free-living, host-tissue and host-biofilm associated microbiomes were assessed and correlated to environmental water quality parameters. Indicator value analysis and machine learning approaches were successfully used to identify microbial indicator taxa as well as to predict coral reef ecosystem health. Our study provides the first framework for integration of microbial based monitoring approaches in coral reef monitoring initiatives.

Cyanate and urea as substrates for marine ammonia oxidation

Katharina Kitzinger^{*1}, Cory C Padilla², Hannah K Marchant¹, Craig W Herbold³, Abiel T Kidane¹, Sten Littmann¹, Philipp F Hach¹, Maria Mooshammer³, Jutta Niggemann⁴, Andreas Richter³, Frank J Stewart², Michael Wagner³, Marcel M M Kuypers¹, Laura A Bristow¹

¹Max-Planck-Institute for Marine Microbiology, Germany, ²Georgia Institute of Technology, United States, ³University of Vienna, Austria, ⁴Carl von Ossietzky University Oldenburg, Germany

In marine systems the first step of nitrification, ammonia oxidation, is mainly performed by highly abundant ammonia oxidizing archaea of the phylum Thaumarchaeota. Thaumarchaeota have generally been considered to be metabolically restricted organisms that only use ammonia as a substrate for energy generation. However, there is evidence that urea and/or cyanate can be used as an alternative ammonia source by some Thaumarchaeota. So far however, it is unclear whether Thaumarchaeota in the ocean directly utilize urea and cyanate, and if utilization of these substrates contributes to their ecological success.

We assessed the use of cyanate and urea by Thaumarchaeota in the eutrophic Gulf of Mexico, an area of intense nitrification activity. We combined biogeochemical rate measurements with molecular and single cell analyses and show that Thaumarchaeota use urea and cyanate for nitrification and N-assimilation. We observed substantial and linear rates of nitrite production from urea and cyanate, with urea- and cyanate-derived oxidation rates ranging between 0.5 to 10% of the ammonia oxidation rates. Furthermore, nanoSIMS analyses of single cell nitrogen uptake derived from ammonium, cyanate and urea revealed that Thaumarchaeota incorporated significantly more N from all these substrates than most of the surrounding cells. Our findings indicate that marine Thaumarchaeota use urea and cyanate to supplement their nitrification and N-assimilation activity, even in ammonium-replete systems. Based on these results we hypothesise that urea and cyanate may also be important substrates for nitrification in ammonium-deplete, oligotrophic waters that characterize much of the ocean.

Contrasting mechanisms shape the sunlit global-ocean microbiome

Ramiro Logares^{*1}, Ina M. Deutschmann², Caterina. R. Giner², Anders K. Krabberød³, Thomas Sebastian Benedikt Schmidt⁴, Laura Rubinat-Ripoll⁵, Mireia Mestre², Clara Ruiz-González², Marta Sebastián², Colomban de Vargas⁵, Silvia G. Acinas², Carlos M. Duarte⁶, Josep M. Gasol², Ramon Massana²

¹Institute of Marine Sciences (ICM-CSIC), Spain, ²ICM-CSIC, Spain, ³University of Oslo, Norway, ⁴EMBL, Germany, ⁵CNRS, France, ⁶KAUST, Saudi Arabia

The smallest members of the sunlit-ocean microbiome (prokaryotes and picoeukaryotes) participate in a plethora of ecosystem functions with planetary-scale effects. Understanding the processes determining the distribution of species composing this assemblage can help us better understand the links between microbiome change and ecosystem function. Yet, the relative importance of the

ecological mechanisms structuring the surface-ocean microbiome were barely known. Ecological theory predicts that the action of selection, dispersal and drift determines the distribution of species, therefore here we quantified the action of these processes on marine microbial assemblages by using community DNA-sequence data from both picoeukaryotes and prokaryotes collected during the Malaspina-2010 expedition. We found that contrasting mechanisms shape the sunlit-ocean microbiome: limited dispersal was the dominant process structuring picoeukaryotic communities, while a combination of limited dispersal, selection and drift shaped prokaryotic counterparts. By investigating associations between environmental parameters with both the structure of communities and individual species-abundances, we determined that the same environmental variables can exert different degrees of selection in picoeukaryotes and prokaryotes. In particular, selection via temperature seemed to influence strongly prokaryotic species co-occurrences, which was not observed in picoeukaryotes. Furthermore, we found that limited dispersal was manifested as stronger distance-decay effects in picoeukaryotes than in prokaryotes. Finally, drift appeared to have larger effects in prokaryotes than in picoeukaryotes. Our work contributes to start connecting ocean microbiome composition, ecological mechanisms and ecosystem function, which can provide insights on the effects of environmental change on the microbiome as well as the consequences of microbiome fluctuation on ecosystem function.

Coping the environmental fluctuations of deep-sea hydrothermal vents with a heat-response centered multi-stress-response network

Xiaopan Ma^{*1}, Weishu Zhao², Hao Leng¹, Jiahao Gu¹, Huahua Jian¹, Yu Zhang¹, Xiang Xiao¹

¹Shanghai Jiao Tong University, China, ²University of Rhode Island, United States

Deep-sea hydrothermal vents, characterized by thermal fluctuations and steep physical-chemical gradients, are hotspots for investigating adaptations of life to extreme and dynamic environments. The hyperthermophilic archaeon *Thermococcus eurythermalis*, isolated from Buaymas Basin, has a broad growth temperature range from 50 to 100°C, and it has also relatively wide growth range on pH (pH4-pH9) and hydrostatic pressure (0.1 MPa- 70MPa). Here we take *Thermococcus eurythermalis* as a model for understanding strategies of life coping with various extreme stresses. The quantitative proteomics of *T. eurythermalis* revealed a set of proteins universally in response to diverse environmental stresses including temperature, hydrostatic pressure, pH and salinity. The network based on correlations of the responding proteins and environmental stresses indicated that heat-responding proteins are the hubs of the global-stress-responding-protein network. Hereby, we evolutionary adapted *T. eurythermalis* to a sub-lethal high temperature for about 660 generations. The growth of the evolved strain was not only increased at high temperature stress, but also improved at pH and pressure stresses, while growth at low temperatures was severe dropped. Genome sequencing of the evolved strain revealed 23 mutations with the mutation rates up to 0.0016 Mb⁻¹·replication⁻¹.

We propose that high temperature is the principal environmental stress hyperthermophiles to address in the environmental fluctuations of deep-sea hydrothermal vents. The living strategies of *Thermococcus eurythermalis* are responding various extreme stresses via one set of universal responding proteins. Moreover, the dynamic genomes provide evolutionary advantages for cells to quick adapt extreme stresses in the changing environments.

Rock-hosted microbial communities possess substantial methane oxidizing potential at geologically diverse marine methane seeps

Jeffrey Marlow^{*1}, Amy Gartman², Sean Jungbluth³, Daniel Hoer¹, Peter Girguis¹
¹Harvard University, United States, ²US Geological Survey, United States, ³Joint Genome Institute, United States

Seafloor methane seeps represent important conduits between subsurface, water column, and atmosphere methane reservoirs, and microbiological consumption of methane is a critical control on the escape of this strong greenhouse gas. At sites of authigenic carbonate formation, carbonate rocks typically comprise >90% of the methane-perfused volume, signifying an enormous potential habitat for endolithic microorganisms enacting the anaerobic oxidation of methane (AOM). Endolithic AOM was previously detected at a single site, but its global relevance has remained uncertain.

Here, we show that endolithic AOM is likely a common phenomenon that could re-configure global methane budgets. Using the newly developed monodeuterated methane technique to quantify oxidation, rates of AOM were determined at seven study sites across a range of depths and geologic contexts. Of particular note, the newly described Point Dume site represents a previously unseen seep setting with highly unusual carbonate “chimney” structures, which, when subjected to *in situ* pressure, produced the highest rates of methane oxidation observed to date. 16S rRNA gene surveys demonstrated clear differences in community structure between sites, while single-nucleotide resolution revealed niche partitioning and syntrophic partnerships in new detail. In-place fluorescence *in situ* hybridization analyses conducted via confocal microscopy preserved microscale spatial associations, offering a realistic view of complex endolithic communities that is typically lost in biomass concentrating procedures.

Given the seemingly pervasive occurrence of endolithic AOM and the elevated rates of methane oxidation we observed, carbonate-hosted methanotrophy signifies an underappreciated but potentially dominant habitat for methane-consuming microbial communities at and below the seafloor.

The mechanism of rapid aggregation behavior in *Trichodesmium*

Ulrike Pfreundt*¹, Anupam Sengupta¹, Martin Ackermann², Roman Stocker¹

¹*Institute for Environmental Engineering, Department of Civil, Environmental and Geomatic Engineering, ETH Zurich, Switzerland,* ²*Department of Environmental Systems Science, ETH Zurich, and Department of Environmental Microbiology, Eawag, Switzerland*

Trichodesmium, multicellular filamentous cyanobacteria, are responsible for ~50% of marine nitrogen fixation. A fundamental aspect of their biology is the formation of aggregates comprising hundreds of filaments, generally distinguished into needle-like tufts, spherical puffs, and bowties. Aggregation and resulting aggregate behavior likely impacts biogeochemical processes, but neither are reasons for the existence of different aggregate morphologies known nor is the mechanism for aggregation established. We hypothesized i) that aggregation itself is due to random encounters aided by turbulent flow, and ii) that aggregate behavior can be tuned and is an emergent property of the gliding motility of *Trichodesmium*. Using custom-built imaging setups, quantitative image analysis, and experimental manipulation of the environment and of cell physiochemistry, we show that aggregates tune their density and morphology in response to environmental cues within minutes, and thus on a response timescale significantly shorter than currently assumed. These responses occur against the backdrop of a clearly defined circadian rhythm in aggregate behavior. We present a minimal model that captures this behavior based on the motility properties of individual filaments. Our analysis also suggests that the current distinction of aggregates into three morphotypes is frail. Instead, individual aggregates transition from one state to the other through collective changes of filament properties and resulting physical interactions. These results shed light on a long-known behavior of one of the biogeochemically most important marine microorganisms, and reveal that properties at the level of the aggregate can emerge from simple behavior of individual filaments, without the need for complex coordination.

Type-3 rhodopsins: A distinct group of microbial retinylidene proteins discovered via functional metagenomics

Alina Pushkarev*¹, Keiichi Inoue², Natalya Yutin³, Eugene V. Koonin³, Itai Sharon⁴, Hideki Kandori², Satoshi Tsunoda², Oded Beja⁵

¹*Mosad HaTechnion, Israel,* ²*Department of Life Science and Applied Chemistry Nagoya Institute of Technology, Japan,* ³*National Institutes of Health, United States,* ⁴*Migal Galilee Research Institute, Israel,* ⁵*Technion – Israel Institute of Technology, Israel*

Many organisms capture or sense sunlight using rhodopsin pigments, including type-1 microbial rhodopsins and type-2 animal rhodopsins. Here, using functional metagenomics, we report a previously unnoticed, diverse family, type-3 microbial rhodopsins, which are distantly related to other rhodopsins and assume the opposite membrane topology compared to type-1 and type-2 rhodopsins, with the N-terminus

facing the cell cytoplasm. Type-3 rhodopsins show photocycles >1 s when expressed in *Escherichia coli*, suggestive of light sensory activity. Photocycles accompany retinal isomerization and proton transfer as in type-1 and type-2 rhodopsins, however protons are never released even transiently. Type-3 rhodopsins are abundant and distributed globally, being detected in soil, freshwater, marine and hypersaline environments, and in psychrophilic, mesophilic and even hyperthermophilic microbes including archaea, bacteria, eukarya and their viruses. Our findings indicate that a previously unaccounted for light-sensing mode commonly occurs in microbes worldwide.

Bacteriophage strategies promoting microbial dominance in coral reefs

Cynthia Silveira^{*1}, Andreas Haas², Antoni Luque¹, Forest Rohwer¹

¹*San Diego State University, United States*, ²*NIOZ, Netherlands*

Marine viruses impact bacterial communities through predation and gene transfer. In coral reefs, increased microbial dominance driven by anthropogenic disturbance lead to a switch in phage replication strategies from lysis, when phages act as predators, to lysogeny, when they become mutualists. Here we investigated the mechanisms driving these shifts and identified metabolic and diversity transitions in the bacterial community leading to decreased phage predation. Fleshy algae release exudates that have low ratio of oxygen-to-dissolved organic carbon (DOC), compared to calcifying organisms. This decoupling between reducing (DOC) and oxidizing (O₂) power leads to metabolic shifts in the heterotrophic microbial community towards biosynthesis, with high biomass yield and low O₂ consumption per cell. Metagenomic analysis indicates a shift in central carbon metabolism that increase cAMP production, the canonical biochemical signal for lysogenic decision in phage lambda. Additionally, bacterial communities in algae-dominated reefs have low diversity, with high dominance of copiotrophs. A mathematical model shows that at low diversity, the probability of encounter between a phage and a suitable host is higher than at high diversity. Frequent successful encounters increase the multiplicity of infection, i.e., the number of phages simultaneously infecting the same cell, the second canonical determinant of lysogeny in lambda. Our analyzes indicate that viral predation represents a resilience mechanism against reef microbialization, and that the switch to lysogeny contributes to the stability of microbialization states in degraded reefs.

Signatures of nutritional conditions in a predatory photosynthetic protist

Susanne Wilken^{*1}, Chang Jae Choi², Avinash Sreedasyam³, Richard Hayes⁴, Jeremy Schmutz⁴, Jerry Jenkins⁴, Jane Grimwood⁵, Kerrie Berry³, Alyson Santoro⁶, Thomas Richards⁷, Ramon Massana⁸, Patrick Keeling⁹, Alexandra Worden²

¹*University of Amsterdam, Netherlands*, ²*Monterey Bay Aquarium Research Institute, United States*, ³*HudsonAlpha Institute for Biotechnology, United States*, ⁴*Joint Genome Institute, United States*, ⁵*HudsonAlpha Institute for Biotechnology, United States*, ⁶*University of California Santa Barbara, United States*, ⁷*University of Exeter, United Kingdom*, ⁸*Institute of Marine Sciences (ICM-CSIC), Spain*, ⁹*University of British Columbia, Canada*

Photosynthetic microbial eukaryotes form the base of many marine food webs. Some protists act both as primary producer and consumer by combining photosynthetic with phagotrophic nutrition in a mixotrophic lifestyle. While the molecular signature of photosynthesis can be detected by the presence of large, conserved and well-studied protein complexes, little is known about the genomic signatures of phagotrophy.

To identify transcriptional patterns associated with different nutritional conditions in mixotrophs, we developed an experimental model system using the marine chrysophyte *Ochromonas* CCMP1393. First, we sequenced its 56Mb genome. Second, we developed an experimental system providing live, fluorescently-labeled bacterial prey with known antibiotic resistance. This allowed controlled manipulation of prey availability, i.e. continuous availability, no prey, or refeeding after no availability. Synchronization of cell division over the diurnal cycle allowed separating direct effects of feeding from factors reflecting progression through the cell cycle.

Of 4,579 differentially expressed genes, 42% were primarily affected by the diurnal cycle, while the other half showed clear responses to prey availability. Among genes with known functions in phagocytosis those involved in prey capture and ingestion showed higher transcript abundances when prey were absent, while transcripts involved in later steps of phagosome processing and digestion were more abundant in actively feeding cells. The observed transcriptional patterns provide the first indicators for detection of nutritional conditions in nature. Ongoing analyses address the massive signal of unknown-function transcripts after prey depletion. Collectively, these results will help dissect the diverse trophic roles of microbial eukaryotes that shape the marine carbon cycle.

Resolving host-phage dynamics at ecosystem scales

Rohit Ghai^{*1}, Vinicius Kavagutti¹, Maliheh Mehrshad¹, Adrian-Stefan Andrei¹,
Michaela Salcher²

¹*Institute of Hydrobiology, Czech Republic*, ²*Limnological Station, University of Zurich, Switzerland*

Phages infect nearly a third of all microbial cells in aquatic habitats. Apart from their ecological role in recycling organic matter and inorganic nutrients, they also modulate microbial lifestyles and evolutionary histories. The close linkages within the microbial community have historically affected our ability to culture both microbes and their phages and major gaps remain in our understanding of host-phage dynamics in natural systems. We used the culture-free metagenomics approach, sequencing epi- and hypolimnion time-series samples from a freshwater reservoir over a period of two years. We assembled more than 2000 near-complete microbial genomes and nearly 3000 complete phage genomes which is the largest genomic collection from freshwaters till date. These genomes represent all major groups of microbial phyla and their phages observed at this single site. Using a combination of different methods (e.g. auxiliary host genes, time-series dynamics), we could predict that many of these phages infect highly abundant freshwater lineages, e.g. Actinobacteria, Betaproteobacteria etc. In the space of multiple seasons, we observed complex phage abundance patterns, e.g. punctuated abundance spikes in the more dynamic epilimnion in contrast to relative plateaus in the more stable hypolimnion. While the microbial genomes at large exhibit a predictable recurrent pattern across multiple seasons, only part of the phage genomes follow their lead. This is seen most remarkably in the hypolimnion where apparently large numbers of phages practically disappear from one winter to the next, indicating an unexpectedly massive turnover in the phage population.

Rapid turnover of viral defense islands drives clonal dynamics in marine microbes

Fatima Aysha Hussain*, Joy Yang, Javier Dubert, Mikayla Murphy, Dave VanInsberghe, Kathryn Kauffman, Martin Polz
Massachusetts Institute of Technology, United States

Viruses are key members of all ecosystems and exert top-down control of microbial populations. By selecting for resistance, viruses are thought to be major drivers of the fine-scale diversity observed among closely related microorganisms. In a 93-day time-series of marine *Vibrio* and co-occurring phages, we identify two nearly clonal populations of *V. lentus* (differentiated by <25 SNPs in the core genome) that are exclusively killed by specific phages. Using transposon-mutagenesis, gene knockouts, and comparative genomics, we show that hosts have identical receptors, but differ in phage-defense islands. The distribution of these islands reflects the hosts' phage predation profiles, suggesting these genes mediate viral specificity. These defense islands are strikingly diverse, containing restriction-modification

systems, recently discovered anti-phage genes, and additional unannotated loci shared among subsets of islands. Moreover, we find highly diverse defense islands correlated to phage predation profiles across all other *Vibrio* species analyzed. We further show by quantifying viral and bacterial clonal abundance across the time-series that the different hosts undergo correlated fluctuations at near constant ~5-fold difference in abundance. These differences appear to be driven by phage properties since phages show an inverse abundance profile suggesting that phages directly control host abundance even at low concentrations in the ocean. Our results overall show that phage defense islands are the most rapidly varying genetic islands, displaying astoundingly high diversity and turnover, and are responsible for clonal-level diversification of bacterial populations.

Exploring viral dark matter in marine sponges

Martin Thomas Jahn^{*1}, Ksenia Arkhipova², Sebastian Markert³, Christian Stigloher³, Tim Lachnit⁴, Laura Rix¹, Lucia Pita¹, Anne Kupczok⁴, Marta Ribes⁵, Bas Dutilh², Ute Hentschel¹

¹GEOMAR Helmholtz Centre for Ocean Research Kiel, Germany, ²Theoretical Biology and Bioinformatics, Utrecht University, Netherlands, ³University of Würzburg, Division of Electron Microscopy, Germany, ⁴Christian-Albrechts-University of Kiel, Germany, ⁵Institut de Ciències del Mar (ICM-CSIC), Spain

Many marine sponges are associated with highly complex and specific microbial communities. As filter-feeding animals, sponges pump up to 24,000 litres of seawater through their system per day, exposing them to up to an estimated $\sim 2.4 \times 10^{13}$ viruses daily. High exposure to phages, a major bacteriolytic element, raises questions on how microbiome homeostasis can be maintained. Moreover, the diversity and function of residual phages on the sponge microbial community and their distribution in the holobiont landscape are largely unexplored. Here, we investigate the DNA/RNA of 36 viromes of four Mediterranean sponge species and nearby seawater references using viral metagenomics. By comparing abundance profiles of 8,135 detected viral scaffolds between sponge populations, species and between individuals we find that sponge viromes are individually unique, host species specific and different to environmental seawater. Only a subset of the viral scaffolds was assigned to known phages indicating that a major part is unknown novel sequence space. We further localised metagenome predicted virions and lysogenic phages within the host mesohyl matrix using a new approach of correlative light and electron microscopy. Overall, this study reveals that phages are an integral and host-specific part of Mediterranean sponge holobionts that may have yet to be identified regulatory roles to maintain microbiome homeostasis.

Discovering tailless viruses in the oceans and in the human body

Libusha Kelly^{*1}, Kathryn Kauffman², Saad Khan¹, Steven Almo¹, Martin Polz²

¹*Albert Einstein College of Medicine, United States*, ²*Massachusetts Institute of Technology, United States*

We recently discovered a novel family of non-tailed dsDNA bacterial viruses that are members of the ancient double jelly roll (DJR) capsid lineage, and showed that operational biases contribute to the systematic loss of such viruses in cultivation- and sequence-based studies. Host range assays of members of this family, the *Autolykiviridae*, on 318 potential Vibrionaceae hosts, revealed that they infected significantly more hosts species than did tailed viruses, which form the basis for current models of virus-host interactions. Using computational approaches to investigate the diversity of DJR lineage viruses we found that, although underrepresented in viral culture and sequence collections, DJRs are present in the genomes of diverse major bacterial and archaeal phyla, as well as in marine water column and sediment metagenomes. Here we use our curated reference set of DJR capsid viruses to identify additional related, previously undiscovered, DJRs in human gut and skin microbiomes and other animal microbiomes. We organize the extensive sequence diversity of DJRs by annotating conserved genes and elements in their genomic neighborhoods in cultured isolates and metagenomic datasets. We describe structural genomics efforts to characterize DJRs from the human microbiome and propose new computational approaches to identify novel DJRs. Our finding that DJR lineage viruses are widespread and genomically diverse in ecosystems as different as the ocean and the human microbiome underscores the need to better define their role in the health and function of ecosystems. More generally, our work identifies new avenues for improved laboratory-based and computational approaches for virus discovery.

A new extensive and curated genome catalogue to enable large-scale viral ecogenomics in freshwater ecosystems

Myriam Labbé^{*1}, Alexander I. Culley¹, Nikos Kyrpides², Emiley Eloë-Fadrosh², Simon Roux²

¹*Centre for Northern Studies (CEN), Université Laval, Canada*, ²*DOE-Joint Genome Institute, United States*

Viruses are abundant and important in freshwater ecosystems as they participate in the regulation of microbial biomass and drive microbial community metabolic output and evolution. Early metagenomic studies have shown that freshwater viruses are distinct from the ones observed in other aquatic environments like the open ocean, however, no large-scale study has yet explored the global diversity of freshwater viruses. Here we present the first attempt to aggregate freshwater virus genomes from next-generation metagenomes using sequences available through the IMG and IMG/VR databases hosted by the Joint Genome Institute (CA, USA) in a single curated and specialized database. Over 9,000 metagenomes and metatranscriptomes have been analyzed and sorted prior to the creation of the database, which now regroups sequences from various freshwater environments

ranging from lakes to rivers and wetlands. This database provides an improved mapping of the freshwater viral sequence space and makes it available for researchers. In addition, this extensive set of metagenomes enables broad-spectrum analysis of the ecology and functional diversity of freshwater viruses, focusing on maximizing functional affiliation through protein clusters analysis, evaluating the distribution of known and novel auxiliary metabolic genes (viral genes of cellular origin that modify the host metabolism during the viral infection) and estimating how these genes may impact the dynamics of microbial communities. The unprecedented scale of this research will open numerous possibilities for freshwater microbial ecologists and contribute greatly to the current knowledge on freshwater viral diversity while providing a foundational resource for future freshwater viral metagenomics and ecogenomics projects.

Expression profiling of host and virus during a coccolithophore bloom provides insights into the role of viral infection in promoting carbon export

Uri Sheyn^{*1}, Shilo Rosenwasser², Yoav Lehahn³, Noa Barak-Gavish¹, Ron Rotkopf¹, Kay Kay⁴, Ilan Koren¹, Daniella Schatz¹, Assaf Vardi¹

¹Weizmann institute of science, Israel, ²The Robert H. Smith Institute of Plant Sciences and Genetics in Agriculture, The Hebrew University, Israel, ³University of Haifa, Israel, ⁴Rutgers University, United States

The cosmopolitan coccolithophore *Emiliana huxleyi* is a unicellular eukaryotic alga that forms vast blooms in the oceans impacting large biogeochemical cycles. These blooms are often terminated due to infection by the large dsDNA virus, *E. huxleyi* virus (EhV). It was recently established that EhV-induced modulation of *E. huxleyi* metabolism is a key factor for optimal viral life cycle. Despite the huge ecological importance of this host-virus interaction, the ability to assess its spatial and temporal dynamics and its possible impact on nutrient fluxes is limited by current approaches that focus on quantification of viral abundance and biodiversity. Here we applied a host and virus gene expression analysis as a sensitive tool to quantify the dynamics of this interaction during a natural *E. huxleyi* bloom in the North Atlantic. We used viral gene expression profiling as an index for the level of active infection and showed that the latter correlated with water column depth. Intriguingly, this suggests a possible sinking mechanism for removing infected cells as aggregates from the *E. huxleyi* population in the surface layer into deeper waters. Viral infection was also highly correlated with induction of host metabolic genes involved in sphingolipid and antioxidant metabolism, providing evidence for modulation of host metabolism under natural conditions. The ability to track and quantify defined phases of infection by monitoring co-expression of viral and host genes, coupled with advance omics approaches, will enable a deeper understanding of the impact that viruses have on the environment.

Mechanisms of biofilm-phage interaction dynamics

Lucia Vidakovic*, Praveen K. Singh, Raimo Hartmann, Carey D. Nadell, Knut Drescher

Max Planck Institute for Terrestrial Microbiology, Germany

In nature, bacteria primarily live in surface-attached, multi-cellular communities, termed biofilms. In medical settings, biofilms cause devastating damage during chronic and acute infections; indeed, bacteria are often viewed as agents of human disease. However, bacteria themselves suffer from diseases, most notably in the form of viral pathogens termed bacteriophages, which are the most abundant replicating entities on Earth. Phage–biofilm encounters are undoubtedly common in the environment, but the mechanisms that determine the outcome of these encounters are unknown. Using *Escherichia coli* biofilms and the lytic phage T7 as models, we discovered that an amyloid fibre network of CsgA (curli polymer) protects biofilms against phage attack via two separate mechanisms. First, collective cell protection results from inhibition of phage transport into the biofilm, which we demonstrate in vivo and in vitro. Second, CsgA fibres protect cells individually by coating their surface and binding phage particles, thereby preventing their attachment to the cell exterior. These insights into biofilm–phage interactions have broad-ranging implications for the design of phage applications in biotechnology, phage therapy and the evolutionary dynamics of phages with their bacterial hosts.

Metatranscriptome data reveal that multifunctional redundancy is controlled to a large extent by the characteristics of community members

Sara Beier*, Dandan Shen, Thomas Schott, Klaus Jürgens
Leibniz Institute for Baltic Sea Research, Germany

The assessment of functional redundancy (FR) of multiple traits is essential to understand community structure-function relationships as FR buffers the functional performance of communities against changes in community composition. In earlier studies FR was estimated by relating differences in community composition to differences in community functioning, but rate measurements are time consuming and only a limited number of functions can be assessed simultaneously. However, meta-omic data can be used to extract information about multiple functional traits from a single dataset. In this study we have developed a metric that quantifies FR based on metatranscriptome data from microbial communities. The magnitude of FR was ranked in samples from a transplant experiment using water characterized by different salinities in which the microorganisms were reciprocally incubated.

The relevance of FR was highest for genes grouped into the category 'Genetic Information Processing' which contains many core genes shared among a high fraction of genomes. In contrast, less FR was detected for genes sorted into the functional categories 'Metabolism' or 'Environmental Information Processing'. Genes in these categories are more specific and in average shared by a smaller fraction of genomes. We also estimated FR between selected groups of taxa, and FR was high between more closely related organisms when communities were grown in similar environmental conditions. Overall, our data revealed a pronounced influence of functional diversity on the one hand but also the characteristics of individual community members on FR, which was specifically high in those communities whose members were more sensitive to salinity changes.

Zooming in to test theoretical assumptions in ecological stoichiometry – C:N:P:K:Ca ratios, homeostasis and demands in saprotrophic soil fungi

Tessa Camenzind*, Matthias C. Rillig
Freie Universität Berlin, Germany

Ecological stoichiometry represents a powerful tool to understand and predict biochemical processes at different scales. Differences among soil, plant and microbial element ratios are used to predict nutrient limitations, element cycling and ultimately the fate of carbon in soils. These seminal applications are based on fundamental assumptions about soil microorganisms, which have rarely been tested at the level of individual species. This is especially the case for saprotrophic fungi, which substantially contribute to nutrient recycling in soils. Thus, we tested hypotheses derived from those theoretical assumptions that (1) stoichiometric ratios of fungi in soil are relatively homogeneous, mainly depending on phylogeny, (2)

unlike autotrophs soil fungi show strong homeostasis especially regarding C:N ratios and (3) species-dependent patterns of nutrient stoichiometry can be used as an ecological trait for soil fungi, which predicts growth patterns and nutrient demands. We used a well characterized fungal collection of 31 species isolated from a temperate grassland. Natural C:N:P:K:Ca ratios of individual species were assessed on soil-extract medium. In parallel, optimal values of those ratios were determined as a proxy for nutrient demands, and responses to deviations in C:N and C:P from optimum values were analyzed with respect to biomass production, mycelial growth and stoichiometric homeostasis. The fungi differ in a wide range of architectural, physiological and ecological attributes, and these novel traits add detailed functional specifications related to nutrient cycling. Additionally, direct correlations of stoichiometry, nutrient limitations and carbon use efficiency at the species level allow us to evaluate fundamental assumptions in ecological stoichiometry.

A high diversity of differently distributed mixotrophic protists is sustaining the ubiquity of mixotrophy in the global Ocean

Emile Faure^{*1}, Fabrice Not², Anne-Sophie Benoiston³, Lucie Bittner³, Sakina-Dorothee Ayata¹

¹*Sorbonne Université, CNRS, Laboratoire d'océanographie de Villefranche, France,* ²*Sorbonne Université, CNRS, Laboratoire Adaptation et Diversité en Milieu Marin, Station Biologique de Roscoff, France,* ³*Sorbonne Université, CNRS, Institut de Biologie Paris Seine, France*

Mixotrophy, or the ability to acquire resources from both auto- and heterotrophy, is an ecological trait that has been shown to be widespread in marine protists. In the last decade, the dichotomy between phyto- and zooplankton has started to disappear to give way to a continuous vision between a state of full heterotrophy and one of full autotrophy. Hence, mixotrophic organisms should always be considered when it comes to estimating primary production, and their population dynamics should be included in marine ecosystems models. For that, a better understanding of the biotic and abiotic factors that shape their distributions is necessary. Here, using a metabarcoding dataset of plankton from the global ocean, we identified a set of 140 taxonomic lineages classified into four mixotrophic functional types. We confirmed that mixotrophy is ubiquitous and detected generalist non-constitutive mixotrophs in new zones compared to previous morphological studies. We showed that constitutive and non-constitutive mixotrophs share similar global distributions, even though not always dominating in similar environmental conditions. Some lineages displayed strongly opposed distributions, across but also within mixotrophic types. Particularly, very divergent biogeographies were found within endo-symbiotic non-constitutive mixotrophs. We propose that the ability to form colonies, as well as the mode of symbiosis are traits playing an important role on the distribution of these organisms. Our results highlight how metabarcoding can complement morphological observations to study the biogeography of protists and identify key drivers of their biogeography. This work will facilitate the integration of under-regarded functional groups into ecological modeling studies.

Meta-analysis of community sequencing datasets reveals a key role of generalist species in microbial dispersion and evolution

Wataru Iwasaki*, Sira Sriswasdi, Ching-chia Yang
The University of Tokyo, Japan

Microbes form fundamental bases of every Earth ecosystem. As their key survival strategies, some microbes adapt to broad ranges of environments, while others specialize to certain habitats. While ecological roles and properties of such "generalists" and "specialists" had been examined in individual ecosystems, general principles that govern their distribution patterns and evolutionary processes have not been characterized. Here, we thoroughly identified microbial generalists and specialists across 61 environments via meta-analysis of community sequencing data sets and reconstructed their evolutionary histories across diverse microbial groups using the Binary-State Speciation and Extinction (BiSSE) model (Sriswasdi, Yang, and Iwasaki. *Nat. Commun.*, 8, 1162. (2017)). This revealed that generalist lineages possess 19-fold higher speciation rates and significant persistence advantage over specialists. Yet, we also detected three-fold more frequent generalist-to-specialist transformations than the reverse transformations. These results support a model of microbial evolution in which generalists play key roles in introducing new species and maintaining taxonomic diversity.

Trait-based life-history strategies explain succession of complex bacterial communities under varying disturbance

Ezequiel Santillan*, Hari Seshan, Florentin Constancias, Stefan Wuertz
Singapore Centre for Environmental Life Sciences Engineering (SCELSE) / Nanyang Technological University, Singapore

Functional tradeoffs are expected after community perturbations since organisms reallocate resources to facilitate recovery. Microorganisms could then adopt distinct life strategies across a disturbance range, which has been suggested but not yet verified. Here we analyzed the effect of disturbance on the assembly, functionality, and distribution of traits in activated sludge bacterial communities, to identify community life-strategies using an ecological trait-based framework.

Sequencing batch microcosm bioreactors (20 mL working volume) were inoculated with activated sludge from a full-scale plant and operated for 35 days. The daily complex feed included toxic 3-chloroaniline (3-CA) at varying frequencies. Eight levels of disturbance in triplicate independent reactors (n = 24), received 3-CA every day (press-disturbed), every two, three, four, five, six, or seven days (intermediately-disturbed), or never (undisturbed). Samples were analyzed by metagenomics, 16S amplicon sequencing, biomass quantification, and chemical characterization of effluent.

After 35 days, bacterial communities displayed significant differences in community structure and composition across disturbance levels, which along with trade-offs in community aggregated function and genotypic traits indicated that communities adopted different life-strategies depending on the disturbance frequency. We semi-quantitatively aligned our results within Grime's CSR theoretical framework, through ordinations and network analysis. Ruderal organisms (R) predominated in communities at intermediately disturbed levels, stress tolerants (S) in press-disturbed reactors, and competitors (C) in undisturbed ones.

We showed that the CSR framework can characterize and assign complex datasets of microbial traits, functions, and taxa into ecologically meaningful components to understand the mechanisms behind the system's response to disturbance.

Single-cell trait-based biodiversity in microbial communities and its link to ecosystem functioning in a stratified lake

Frank Schreiber^{*1}, Simone Fontana², Francesco Danza³, Stephane Escrig⁴, Anders Meibom⁴, Francesco Pomati⁵, Martin Ackermann⁶

¹Federal Institute for Materials Research and Testing, Germany, ²Eawag, Swiss Federal Institute for Aquatic Science and Technology, Dept. of Aquatic Ecology and WSL, Switzerland, ³Supsi, Laboratory of Applied Microbiology, Switzerland, ⁴Laboratory for Biological Geochemistry, School of Architecture, Civil and Environmental Engineering (ENAC), École Polytechnique Fédérale de Lausanne (EPFL), Switzerland, ⁵Eawag, Swiss Federal Institute for Aquatic Science and Technology, Dept. of Aquatic Ecology, Switzerland, ⁶ETH Zurich, Dept. of Environmental Systems Science and Eawag, Swiss Federal Institute for Aquatic Science and Technology, Dept. of Environmental Microbiology, Switzerland

A fundamental question in ecology is how biodiversity affects ecosystem function. Biodiversity is commonly estimated based on genetic variation. We investigated a new approach that defines and measures biodiversity in complex microbial communities. We used the variation in multiple functionally-relevant, phenotypic traits measured in parallel in single cells as a metric for microbial phenotypic diversity. We studied phenotypic diversity and ecosystem functioning throughout different photosynthetic layers dominated by divergent microbial communities in the gradient of Lago di Cadagno. We determined genetic diversity by 16S and 18S amplicon sequencing and bulk ecosystem functioning (photosynthesis). In addition, we determined phenotypic diversity using single-cell technologies such as nanometer-scale secondary ion mass spectrometry (NanoSIMS) correlated with confocal laser scanning microscopy (CLSM) and scanning flow-cytometry. We measured functional trait variation between individuals in ¹³CO₂ fixation, ¹⁵NH₄⁺ uptake, and variation in physio-morphological cell traits, such as cell size, shape, and auto-fluorescence for various pigments related to photosynthesis. We used the

distances between individuals in a multidimensional trait space to derive phenotypic trait-based diversity indices, such as trait richness, trait evenness, and trait divergence. We find that phenotypic trait divergence associates with ecosystem functioning, whereas genetic diversity does not. Including activity-based, single-cell phenotypic measurements with NanoSIMS provided an additional accuracy to the trait-based diversity assessment and allowed us to formulate hypotheses on the mechanisms that shape the correlation between phenotypic diversity and ecosystem function. Together, our results show that phenotypic diversity is a meaningful concept to measure microbial biodiversity and associate it with ecosystem functioning.

The lottery hypothesis across space and hosts

Torsten Thomas^{*1}, Alexandra Roth-Schulze², Catherine Burke², Enrique Zozaya-Valdes², Jose Pintado³, Bernd Wemheuer², Staffan Kjelleberg², Peter Steinberg²
¹University of New South Wales, Australia, ²Centre for Marine Bio-Innovation & School of Biological, Earth and Environmental Sciences, The University of New South Wales, Australia, ³Instituto de Investigaciones Marinas (IIM-CSIC), Spain

Microbial communities assemble with both stochastic (neutral) and deterministic processes with the relative importance of these processes being frequently debated in the field of microbial ecology. One model that unifies these two processes is the so called "lottery hypothesis", which postulates that communities are randomly assembled from pools of taxonomically distinct species (so called "guilds") that have similar ecological functions. This model was initially used to explain the high taxonomic variability, yet similar predicted functionality of microbial communities associated with the surface of the seaweed *Ulva australis*. However it is not known, if this model is robust across biogeographic gradients or applicable to other host systems. Using metagenomic analyses, we observed that 70% of the *Ulva*-specific predicted functionality was consistently found across large biogeographic scales (i.e. Australia versus Spain), while the remaining functions (~30%) are possibly involved in local adaptations. In contrast to the high level of functional similarity, only one low abundance OTU was common across all samples analysed. When comparing communities of other hosts (various seaweeds and seagrasses) with each other, we further found that taxonomic diversity was unique to each type of host, but that the majority of functions could be found in any given surface community, suggesting a high degree of functional redundancy. Together these observations support a model, whereby communities on marine surfaces are assembled from guilds of microorganisms with a functionality that is partitioned into general properties for a surface-associated life-style, but also specific features that mediate host-specificity.

Multi-omics analysis of uranium stress response in environmental *Microbacterium* isolates

Nicolas Gallois^{*1}, Béatrice Alpha-Bazin², Philippe Ortet¹, Mohamed Barakat¹, Justine Long¹, Laurie Piette¹, Séverine Zirah³, Alain Paris³, Catherine Berthomieu¹, Jean Armengaud², Virginie Chapon¹

¹CEA, CNRS, Aix-Marseille Université, UMR 7265

BVME, France, ²CEA/DRF/Joliot/DMTS/SPI-Li2D UMR CEA-INRA

0496, France, ³MCAM, UMR 7245 CNRS/Muséum National d'Histoire Naturelle, France

Four *Microbacterium* strains have been isolated from radionuclide- and metal-rich environments. They exhibited contrasted uranium-tolerance capabilities, from highly tolerant to sensitive. Cells of *Microbacterium* strains exhibited three sequential mechanisms at 0.5, 4 and 24h uranium exposure: a rapid metal removal from the exposure solution, an active U(VI) release in this solution and an intracellular biomineralization mechanism. While all four strains were able to mineralize U(VI), the efflux mechanism was observed only in the three uranium-tolerant strains. Using a combination of omic approaches, these mechanisms involved in uranium stress response were explored at a molecular level. Metabolomic analysis revealed a clustering of samples by strain, incubation time and treatment. The complete genomes of the four strains were assembled and used to predict the whole proteome. The interpretation of high-throughput proteomic data validated the identification of 1,100 to 2,000 proteins. This innovative proteogenomic approach highlighted that 15 to 25% of the identified proteins were modulated upon uranium exposure. These data show that a complex cellular response to uranium occurs in *Microbacterium* strains. Evidences of uranium influence on iron and phosphate metabolic pathways were obtained. The protein exhibiting the highest positive fold-change in the uranium-tolerant strains has no known function. A combination of biochemical and biophysical methods demonstrate that this protein, named UipA, exhibits a strong affinity for uranium and iron. Moreover, two genes homologous to the metal-resistance two-component system CzcRS were identified upstream the *uipA* gene. These results suggest that UipA is a new uranium-binding protein involved in uranium tolerance.

Biotransformation of hexachlorocyclohexane in biogas reactors: Potential of anaerobic digestion for treatment of contaminated biomass

Shujuan Lian^{*1}, Marcell Nikolausz², Ivonne Nijenhuis², Hans Richnow²

¹Helmholtz Centre for Environmental Research GmbH - UFZ, Germany, ²Helmholtz Centre for Environmental Research - UFZ, Germany

Hexachlorocyclohexanes are persistent organic pollutants with toxic and carcinogenic effect. Although they were banned in 2009, huge amounts of Lindane (γ -hexachlorocyclohexane) had been used before as pesticide worldwide and other isomers as by-products are still found with high concentration in contaminated fields. For clean-up, phytoremediation combined with anaerobic digestion of pesticide-

contaminated biomass to produce biogas could be a promising strategy. To investigate the effect of hexachlorocyclohexanes on the process including potential biotransformation, laboratory-scale batch reactors and continuous stirred tank reactors were set up.

Batch bioreactors with different concentration levels of hexachlorocyclohexanes were used to study the inhibitory effects. Temporal inhibition of acetoclastic methanogens was observed in 150 mg/L set and reductive dehalogenation as major transformation pathway of hexachlorocyclohexanes were identified via isotopic fractionation approach. Benzene and chlorobenzene were identified as metabolites in hexachlorocyclohexanes added reactors. These metabolites had no inhibitory effects on methanogenesis, and after longer incubation they even positively influenced the overall methane yield via promoting disintegration of the lignocellulosic substrate. In addition, 15 L continuous stirred tank reactors were operated for one year by adding hexachlorocyclohexane isomers (γ , α and β) consecutively during anaerobic digestion. No influence on conventional reactor parameters (methane yield, concentration of volatile fatty acids, pH, total ammonia nitrogen etc.) was observed indicating the lack of inhibition. γ and α isomers were transformed to chlorobenzene and benzene and transformation rates increased along with time due to the adaptation of microbial community. Hence, anaerobic digestion appears to be potential application for treatment of hexachlorocyclohexanes contaminated biomass.

The plant microbiome: From ecosystem functioning to biotechnological utilization

Christina Andrea Müller*¹, Stephanie Nadine Hollauf¹, Melanie-Maria Obermeier², Gabriele Berg¹

¹Graz University of Technology / Environmental Biotechnology, Austria, ²Austrian Centre of Industrial Biotechnology, Austria

Sphagnum mosses are colonized by a diverse microbiome. An inter-environmental comparison revealed that the microbiome harbours specific genetic features, which support abiotic stress protection, interaction between microorganisms, nutrient supply and pathogen defence. These essential traits allow it to function sustainably in association with the host plants. Based on these findings we explored the genetic pool of the moss microbiome using functional metagenomics, and decided to tackle two major environmental problems: the growing pollution of the ecosystems with plastic waste, and the rapid and uncontrolled development of antibiotic resistances.

We searched for natural products like antimicrobials produced by nonribosomal peptide synthetases in a *Sphagnum* moss metagenomic clone library. Applying a PCR-based screening we detected 13 clones containing novel synthetase sequences. One synthetase was predicted as a membrane-bound protein that shows structural similarity to homopoly(amino acid)-producing synthetases. Homopoly(amino acid)s are biopolymers of bacterial origin with antimicrobial activity.

A second clone showed antagonistic activity against gram positive bacteria and yeast, suggesting the availability of a lipopeptide-type antibiotic.

A screening for enzymes with the ability to hydrolyze synthetic polyesters was also successful. We cloned and characterized six novel esterases, one of them showing outstanding hydrolytic activity for degradation of the employed polyester foil. The newly discovered enzymes have the potential to be applied in polyester recycling processes.

Our metagenomic studies provide insights into the molecular diversity of the moss microbiome, which not only protects the plant in the bog ecosystem but also provides a valuable source for the discovery of bioactive molecules and enzymes.

Microbial response and functional resilience against the toxicity of engineered polymeric nanoparticle

Susmita Das Nishu*, Seung Bin Park, Yuhyun Ji, Han Il, Jaehong Key, Tae Kwon Lee
Yonsei University, South Korea

Poly(lactic-co-glycolic acid) (PLGA) is a widely adopted, polymer-based, engineered nanoparticle used in drug delivery system because of its attractive features of biodegradability and biocompatibility. Degradation time of PLGA nanoparticles can vary from several months to years, which is arising concern about their potential ecotoxicity. Cognitive knowledge of PLGA toxicity on soil microbial function and community structure is confined, unlike metal nanoparticles. This study aimed to investigate the impact of PLGA nanoparticles with different surface charges (positive and negative) on nitrification and microbial community in the soil environment. Two nitrifying bacteria, *Nitrosomonas europaea*, and *Nitrospira moscoviensis*, were treated with different concentrations of PLGA nanoparticles. Above 0.01 mg/mL of both charged nanoparticles inhibited nitrification. Although the inhibitory effects of the charge and treatment time on nitrification varied in nitrifying bacteria, the nitrification rate in both bacteria was partially recovered after 14 days. But surprisingly the nitrification rate in nanoparticle treated soil sample was fully recovered after 14 days. Regardless of the clear recovery of nitrification, the soil microbial communities were significantly shifted by the nanoparticles within the same treatment time. We found a time-based community shift, as well as alteration in microbial community structure, was greatly influenced by incubation time, rather than the soil type or surface charge of nanoparticles. The toxicity of PLGA nanoparticles directly affected the nitrifying activities in the soil environment within a short period, and the effects dissipated with time. This study uplifted new avenues of research to regulate ecotoxicity and environmental hazard of a biocompatible polymeric nanoparticle.

The potential for polyphosphate cycling in Archaea and extensive stress-induced polyphosphate formation in *Methanosarcina mazei*

Fabiana Paula^{*1}, Jason Chin², Anna Schnurer³, Bettina Muller³, Panagiotis Manesiotis⁴, Katrina Macintosh², Jasmine Connolly⁵, Florence Abram⁵, John McGrath², Vincent O'Flaherty⁵

¹Microbiology, School of Natural Sciences, National University of Ireland Galway, Ireland, ²School of Biological Sciences and the Institute for Global Food Security, Queen's University Belfast, United Kingdom, ³Microbiology, Swedish University of Agricultural Sciences, Sweden, ⁴School of Chemistry and Chemical Engineering, Queen's University Belfast, United Kingdom, ⁵Microbiology, National University of Ireland Galway, Ireland

Many bacteria are known to store phosphorus as polyphosphate under aerobic conditions, a trait already exploited for phosphorus removal during wastewater treatment. By contrast, few reports detail the presence of polyphosphate in Archaea, with little information available regarding its function, environmental triggers, metabolic turnover, and associated gene regulation. Furthermore the mechanisms involved in anaerobic polyphosphate accumulation are not well understood. Here we report that homologs of key bacterial polyphosphate cycling genes are present across the major taxa in the Archaea including a remarkable diversity of methanogens. In addition we describe, for the first time, how polyphosphate accumulation can be induced in a methanogen in response to extreme oscillation in phosphate availability, applying “polyphosphate overplus” approach i.e. phosphate starvation, followed by incubation in high phosphate media. Exposure of *Methanosarcina mazei* to these conditions resulted in i) the production of polyphosphate-like intracellular granules, ii) a 200% increase in cellular P content and iii) a >3.7 fold increase in polyphosphate kinase gene transcripts. Additionally, under phosphate starvation, genes for alkaline phosphatase and for high affinity phosphate specific transport (Pst) system were overexpressed. Together these results demonstrate that polyphosphate gene homologs are widespread amongst the Archaea, and that their expression can be induced by changes in phosphorus availability in a similar manner to that observed in bacteria. Such anaerobic polyphosphate metabolism raises questions about the impact of archaeal phosphorus cycling in both engineered and natural systems, especially under low oxygen conditions.

The antimicrobial properties of nitrous acid and its use to control bioprocesses in engineered environments

Nathan Todd^{*1}, Shu-Hong Gao², Andrew Laloo¹, Lu Fan³, Jun-Yuan Ho¹, David Richardson⁴, Lai Peng⁵, Jianhua Guo¹, Miriam Agullo-Barcelo¹, Justin Wei¹, Dongbo Wang⁶, Sharman Narayanasamy⁷, Inka Vanwonterghem⁸, David Waite⁹, Jason Steen⁹, Anne Kaysen⁷, Anna Heintz-Buschart⁷, Paul Wilmes⁷, Qilin Wang¹⁰, Benjamin Schulz¹¹, Amanda Nouwens¹¹, Philip Hugenholtz⁹, Zhiguo Yuan¹

¹The University of Queensland / Advanced Water Management Centre, Australia, ²University of Oklahoma, Institute for Environmental Genomics, United States, ³iCarbonX, China, ⁴University of East Anglia / School of Biological Sciences, United Kingdom, ⁵Ghent University, Belgium, ⁶Hunan University, China, ⁷University of Luxembourg, Luxembourg, ⁸The University of Queensland, Australia, ⁹The University of Queensland / Australian Centre for Ecogenomics, Australia, ¹⁰Griffith University, Australia, ¹¹University of Queensland / School of Chemistry and Molecular Bioscience, Australia

Nitrous acid (NA), the protonated form of nitrite (HNO_2) can stress microorganisms and be used to manipulate microbial transformations in wastewater treatment. NA can be applied to protect sewers from the costly microbial concrete corrosion, and for improved nitrogen removal in activated sludge. There are various hypotheses to explain the antimicrobial effects of NA. However, improved applications of NA require better understanding of its antimicrobial mechanisms. In pure cultures of *Pseudomonas aeruginosa* and *Desulfovibrio vulgaris* (denitrifying bacteria (DB) and sulfate reducing bacteria (SRB) respectively) and in mixed microbial communities of nitrifying sludge we examined growth, physiological, transcriptomic, metagenomic and (meta)proteomic responses during exposure to sub-biocidal levels of NA. In the DB and SRB NA was seen to severely inhibit growth, disrupt respiratory mechanisms and shut-down ribosome activity. In the nitrifying sludge high levels of NA (1.82 mg/L NA-N) were applied to suppress the activities of nitrite oxidising bacteria, while ammonia oxidising bacteria (AOB) remained active. In contrast to DB and SRB, during NA exposure AOB displayed increased levels of respiratory proteins and no evidence of ribosome dormancy. Oxidative stress was evident in the SRB and AOB, but not in the DB, and this is potentially a disruptive element to various cell components and activities. Various mechanisms for detoxification of nitrite were observed in these bacteria. Consequently, we find there are multiple antimicrobial effects of NA and these differ between the different bacterial types. This is important to consider during application of NA for control of microbial transformations in environmental biotechnology.

Single-cell phenotyping of functionally relevant populations in full-scale enhanced biological phosphorus removal systems using Raman spectroscopy

Dongqi Wang^{*1}, Nicholas Tooker², Guangyu Li², Varun Srinivasan², Annalisa Onnis-Hayden², April Gu³

¹Xi'an University of Technology, China, ²Northeastern University, United States, ³Cornell University, United States

The advancement of molecular and "-omics" technologies provides great insights into the phylogenetic diversity of microbial community in enhanced biological phosphorus removal (EBPR) systems, but little is known for the phenotypic functions and versatility within phylogenetic groups due to the lack of feasible tools. Here, we applied Single cell Raman spectroscopy (SCRS) for phenotyping and metabolic characterization of functionally relevant populations, namely polyphosphate accumulating organisms (PAOs) and glycogen accumulating organisms (GAOs), in eight full-scale EBPR systems with different configurations, including four side-stream S2EBPRs that host a RAS or MLSS fermentation reactor and four conventional EBPRs. SCRS not only allows for identification of PAOs and GAOs based on their signature intracellular polymers (polyP, PHA and glycogen) fingerprint but also reveals the heterogeneity and intensity distribution of the polymers in individual cells among PAO and GAO populations separately. The intracellular polyP and PHA peak intensity of individual PAO was statistically higher in S2EBPR plants than those in conventional configurations, revealing the underlying mechanism for improved performance and stability in S2EBPR that are associated with higher polyP storage level per cell and higher energy and carbon source (PHA) available for P uptake in the aerobic phase. In addition, the Raman fingerprints of PAOs and GAOs that capture both functionally relevant intracellular polymers and other cellular metabolic traits enable phenotyping and clustering of these key population groups. The phenotyping outcome can be further correlated with both phylogenetic structures and system function parameter matrix to elucidate phylogeny-function relationships.

A comprehensive analysis of the response of the soil microbiome to organic matter content, soil temperature, and soil moisture

Robyn Barbato*, Robert Jones, Karen Foley, Lauren Waldrop, Sergey Vecherin, Komi Messan, John Eylander
US Army ERDC - Cold Regions Research and Engineering Laboratory, United States

Environmental properties influence biological activity and collectively define the state of soils, yet these properties are typically described through time-intensive, ground-based sampling efforts. Techniques have been developed to assess microbial community structure and function in soils; however, questions remain regarding which environmental data are most relevant and how and to what degree their fluxes would impact the system. Our aim was to investigate the soil microbiome's response to soil particle size distribution, temperature, and water potential because they are well established factors that influence microbial activity and could be predicted using remote sensing data and weather forecasts. We conducted an extensive investigation subjecting four soils to a range of temperatures and moistures, and measured their activity through CO₂ efflux and resolved microbial community shifts through amplicon sequencing. Our results showed changes in CO₂ efflux rates according to organic matter content and dynamic conditions. We developed a stochastic model to characterize soil activity under environmentally relevant conditions. Additionally, we resolved bacterial community shifts that were more distinct in soils with limited nutrients, suggesting a buffering capacity inherent in high nutrient soils. The fungal patterns differed in that their response to temperature and moisture was similar regardless of the soil physiochemical properties. These findings have broad implications for modeling microbial activity in soils and resolving patterns in microbial taxonomy.

Soil, climate, and vegetation drivers of soil bacterial abundance: linking niche and phylogeny to predict future soil microbial ecosystem services

Andrew Bissett*¹, Steven D. Mamet², Eric G. Lamb³, Steven D. Siciliano²
¹CSIRO Plant Industry, Australia, ²Department of Soil Science, University of Saskatchewan, Canada, ³Department of Plant Sciences, University of Saskatchewan, Canada

The composition and function of soil microbial communities has been the subject of much research, but the principles underlying the assembly and structure of these complex communities remain incompletely understood. Processes that shape the soil microbiome are thought to be at least partially niche-driven and are thus conceptually amenable to modelling and prediction given soil environmental (niche) information. We employed structural equation and extended Huisman–Olf–Fresco (eHOF) models to describe the niches, niche optima, and broad ecological types of bacteria from 1381 Australian soils. Soils were Illumina 16S rRNA sequenced as part of the Biomes of Australian Soil Environments (BASE) project. Bacterial abundance responses to environmental gradients were usually non-linear and often displayed specific optima. Niche optima were usually taxonomic level-specific, with many bacterial environmental traits requiring complex genetic underpinning (salinity,

pH, water availability) appearing to be phylogenetically conserved. Niches were then used as environmental traits to predict the likely impacts of short term (30 year) climate change scenarios on soil bacterial groups and functional traits (N, C cycling), demonstrating the utility of using response traits derived from links among climate, vegetation, and soil to predict potential changes in ecosystem function. Changes in predicted distributions of functional genes were clearly related directly to climate variations affecting water availability and temperature as well as indirectly to changes in overlying vegetation communities.

Robot-assisted microfluidic cultivation of multispecies biofilms

Tobias Kabbeck*, Silla H. Hansen, Susanne Krause, Carsten P. Radtke, Christof M. Niemeyer, Johannes Gescher
Karlsruhe Institute of Technology, Germany

Biofilms are the main lifestyle of microorganisms, containing great potential in biotechnological processes and coming more and more into focus of scientific attention. Despite the increasing knowledge in this topic, there is still a great complexity to analyze multispecies consortia. Overcoming the problem, we designed a new microfluidic chip-system with easy modifiable cultivation conditions and established a broad spectrum of appropriate analytical tools. The total machine-assisted platform contains the capability of automated FISH-/CARD-FISH and OCT analysis, a μm -accurate online sampling for spatiotemporal resolution imaging and anaerobic cultivation with various gas mixtures. Due to this adjustable chip-setup, we are able to generate and monitor biologically and physically induced gradients.

Based on our special possibilities we aim to separate naturally three-dimensional growing biofilms into fractions allocated over the fluidic channel, with an overarching goal of co-culture-depending enrichment of so far uncultured microorganisms. Via validation experiments with different natural and synthetic mixed cultures, we showed the desired effect of self-organized separation. For instance, we described for the first time a protective co-culture behavior between *Leucobacter chromiirens* and *Escherichia coli*. We were able to show this dependency via online monitoring 16S analysis coupled to downstream FISH evaluation. Furthermore, we enriched the first stable co-culture of an uncultivated member of the Thermoplasmatales and a nanoorganism belonging to the ARMAN (Archaeal Richmond Mine Acidophilic Nanoorganisms). Evolving the enrichment-based usage of our chips, we conducted the first experiments to structure the system as screening platform for the search and optimization of novel strains for biotechnological applications.

Modelling bacteria-phage interactions driving predation and horizontal gene transfer of antibiotic resistance genes

Jorge Moura de Sousa*, Eduardo Rocha
Institut Pasteur, France

Bacteriophages shape microbial communities through predation but also by promoting horizontal gene transfer, including the transmission of pathogenic traits like antibiotic resistance. Here we present a novel individual-based modelling approach to study the evolutionary consequences of bacteria-phage interactions. Our model integrates several important processes involved in the ecological and evolutionary interactions between bacteria and phages, including population dynamics, multiple selective pressures, environmental structure, explicit genome evolution, and phage-mediated horizontal transfer.

We simulate dynamics of experimental evolution within diverse ecological conditions, linking these two scales in microbial communities. We recapitulate experimental and theoretical observations of bacteria-phage population and evolutionary dynamics, while providing novel insights. In particular, we find that structured environments, especially if antibiotics are heterogeneously distributed, promote the acquisition of resistance to both phages and antibiotics. We are also able to quantify the relative importance of different mechanisms (e.g., lysogeny and transduction) for the phage-mediated transmission of antibiotic resistance genes. Importantly, the explicit modelling of bacterial and phage genomes allows to temporally follow the genomic composition of microbial populations. We compare these patterns with observations by comparative genomics of thousands of bacteriophage genomes and their hosts, identifying the mechanisms more likely to drive the spread of resistance genes across bacterial species.

The mechanistic-based theoretical approach we propose is inspired by results from experimental approaches and comparative genomics, but it can also guide further research in these different areas. Such integrative approaches will be fundamental to disentangle the patterns of adaptation in microbial communities and their consequences for public health.

Rapid inference of large-scale ecological networks from heterogeneous microbial sequencing data

Janko Tackmann*¹, João Rodrigues², Christian von Mering²
¹*University of Zürich, Switzerland*, ²*University Zürich, Switzerland*

The recent explosion of metagenomic sequencing data makes tools for rapid computational analysis essential. While such software is becoming increasingly available for OTU mapping and clustering, co-occurrence-based prediction of microbial interactions is lagging behind. Simple correlation-based tools scaling to large data sets exist, yet these do not distinguish between direct and indirect

interactions, resulting in excessive numbers of false positives. Approaches with better resolution, on the other hand, are so far highly limited in the size of data sets they can process. Furthermore, environmental and technical factors, though important confounders for microbial association signals, are rarely considered.

We present FlashWeave, a new software tool that adopts a flexible machine learning framework based on Probabilistic Graphical Models to infer highly resolved microbial interactions from heterogeneous, large-scale microbial abundance data sets. It further provides seamless integration of environmental and technical factors.

FlashWeave is highly optimized for speed, scaling to tens of thousands OTUs and samples, outperforming five state-of-the-art methods by up to three orders of magnitude in run time. It furthermore surpasses current methods in accuracy benchmarks on eight synthetic data sets. We applied FlashWeave to a meta-data set of 69 818 publicly available human gut samples, resulting in one of the largest and most diverse models of microbial interactions in the gastrointestinal tract to date. The network reveals strongly structured sub-communities and wide-spread competition, with a surprising number of negative hub OTUs.

FlashWeave paves the path towards a deeper and more integrated understanding of global interaction trends in microbial ecology.

Microcalorimetric analyses of microbial energy partitioning between growth and maintenance under optimal and suboptimal environmental conditions

Frederick von Netzer*¹, Kristopher A. Hunt¹, Drew Gorman-Lewis², Everett Shock³, Serdar Turkarslan⁴, Christina E. Arens⁴, Anne W. Thompson⁵, Nitin S. Baliga⁴, Aifen Zhou⁶, Jizhong Zhou⁶, Jessica Hardwicke¹, Chiachi Hwang⁷, Matthew W. Fields⁷, Antonio Paiva⁸, Giovanni Pilloni⁸, David A. Stahl¹

¹University of Washington, Department of Civil and Environmental Engineering, United States, ²University of Washington, Department of Earth and Space Sciences, United States, ³Arizona State University, School of Earth and Space Exploration and School of Molecular Sciences, United States, ⁴Institute for Systems Biology, United States, ⁵Portland State University, College of Liberal Arts and Sciences, United States, ⁶University of Oklahoma, Institute for Environmental Genomics, United States, ⁷Montana State University, Center for Biofilm Engineering, United States, ⁸ExxonMobil Research and Engineering, United States

Maintenance energy is a key concept relating microbial growth to energy dissipation. The energy extracted from environmentally available nutrients is partitioned between growth and non-growth associated maintenance (GAM/NGAM). Since nutrient availability and environmental conditions are generally sub-optimal, quantifying the investment in GAM/NGAM is essential to develop a predictive understanding of microbial community assembly and functioning. However, quantifying GAM/NGAM is still elusive, partly due to requiring continuous culture setups to derive maintenance, causing an uncertainty of its relevance as a quantitative parameter. Thus, simplifying the quantification of maintenance could enhance the understanding of community

functioning in complex environments. Therefore, we used microcalorimetry as a direct and sensitive method to quantify the heat per biomass produced in batch cultures as a comparative proxy for maintenance. We applied this approach to different growth scenarios: osmotic stress, simulated environmental dynamics and biocorrosion, linked to molecular and metabolic analyses assessing the energy-demanding costs of adaptation and regulation. For a detailed analysis of NGAM/GAM partitioning, batch cultures of *Desulfovibrio alaskensis* G20 were grown at different temperatures to model changes in GAM/NGAM with increasing temperature. This model extended prior modeling established for chemostats to incorporate microcalorimetric measurements and associated growth chemistry analyses. The new model predicted the maximum growth temperature based on modeled NGAM upturn associated with increasing temperature stress. Optimizing this approach should lead to a quantitative, thermodynamic-based understanding of microbial fitness and competitiveness within their communities.

Bacterial pulmotypes reflect functional microbial processes and associate with clinical outcomes in cystic fibrosis airways

Stefanie Widder^{*1}, Zhao Jiangchao², Qingyang Zhang², Lisa Carmody³, Bridget Foster³, Linda Kalikin³, Mary Rogers⁴, Patrick Schloss⁵, John Lipuma³

¹Medical University of Vienna, Austria, ²University of Arkansas, United States, ³University of Michigan / Medical School / Department of Pediatrics and Communicable Diseases, United States, ⁴University of Michigan / Medical School / Department of Internal Medicine, United States, ⁵University of Michigan / Medical School / Department of Microbiology and Immunology, United States

The complex lung microbiome in persons with cystic fibrosis is an ideal system for establishing principles of emergent, microbial community function interfacing human immunity: decade-long clinical observation provides high-resolution data. With the objective to connect microbial community composition, microbial metabolism and clinical outcomes, we analyzed a longitudinal large-scale data set comprised of bacterial 16S rRNA gene amplicons derived from CF sputum samples (N=818, 109 patients). We exploited hidden data structure by a Dirichlet multinomial mixture model and identified eight alternative microbiome types, termed pulmotypes. These exhibit recurrent transition preferences in patient trajectories and correlate with disease state and stage. We constructed pulmotype-specific activity profiles and found that pulmotype microbiota drive distinct community functions including mucus degradation or increased acid production. As a result of community composition and metabolic activity, the immune system is exposed to selective trigger profiles and responds in differentiated manner, i.e. baseline symptoms or pulmonary exacerbations. We conclude that the pulmotypes are the result of ordered, underlying drivers such as predominant metabolism, ecological competition and niche construction and can form the basis for quantitative, predictive models supporting clinical treatment decisions.

The phylogenetic core; a new conceptual framework applied to the study of the human gut microbiome

Daniel Aguirre de Carcer
Universidad Autónoma de Madrid, Spain

The complex community of microbes living in the human gut plays an important role in host wellbeing. However, defining a 'healthy' gut microbiome in terms of composition has remained an elusive task, despite its anticipated medical and scientific importance. In this regard, a central question has been if there is a 'core' microbiome consisting of bacterial groups common to all healthy humans. Recent studies have been able to define a compositional core in human gut microbiome datasets in terms of taxonomic assignments. However, the description of the core microbiome in terms of taxonomic assignments is not useful when considering subsequent analyses and applications. Through the implementation of a dynamic clustering approach in the meta-analysis of comprehensive 16S rRNA marker gene datasets, this study found that the human gut pan-microbiome presents a preeminent compositional core comprised of discrete units of varying phylogenetic depth present in all individuals studied. Since both microbial traits and ecological coherence show signs of phylogenetic conservation, this outcome provides a new conceptual framework in the study of the ecosystem, as well as important practical considerations which should be taken into account in future research.

Model gut consortia elucidate collaborative multispecies bile acid metabolism

Hans Bernstein^{*1}, Sydney Dautel², Zehra Esra Ilhan³, Rosa Krajmalnik-Brown³, Janet Jansson⁴, Aaron Wright⁴, Thomas Metz⁴

¹*The Arctic University of Norway, UiT, Norway*, ²*University of Washington, United States*, ³*Arizona State University, United States*, ⁴*Pacific Northwest National Laboratory, United States*

Bile acids are metabolic links between hosts and their gut microbiomes, yet little is known about the roles they play in microbe-to-microbe interactions. We performed a study designed to investigate the effect that a common probiotic, *Lactobacillus acidophilus*, has on microbial interactions that lead to formation of secondary bile acids. A model microbial consortium was built from three human gut microbes, *Clostridium scindens*, *Collinsella aerofaciens*, and *Blautia obeum*, and cultured under different bile acid treatments. A multi-omics platform that included mass spectrometry-based metabolomics and activity-based proteomic probes was then used to produce two major results. The first, was that an uncommon secondary bile acid – ursocholate – was produced by a multi-species chemical synthesis pathway. This result highlights a new microbe-to-microbe interaction mediated through bile acids. The second finding was that the probiotic strain, *L. acidophilus*, quenched the observed multi-species interactions and effectively halted consortial synthesis of ursocholate. Little is known about the role that ursocholate plays in human health and development. However, the presence of ursocholate in humans was contextualized in a clinical study that investigated ursocholate abundances related to

gastric bypass surgery. The abundance of ursocholate corresponded with the successful weight loss of patients after gastric bypass surgery, highlighting that it may be important to investigate the implications of patient- and microbe-derived metabolites. More broadly, this study supports an emerging theme in microbiome sciences that microbial interactions are context dependent and that the presence or absence of select species and/or metabolites can have a strong effect on the overall function.

Synthesis of multi-omic data and community metabolic models reveals insights into the role of hydrogen sulfide in colon cancer

Nicholas Chia^{*1}, Vanessa Hale², Patricio Jeraldo³, Michael Mundy³, Janet Yao³, Gary Keeney³, Nancy Scott³, Heidi Cheek³, Jennifer Davidson³, Megan Green³, Christine Martinez³, John Lehman³, Chandra Petty³, Erica Reed³, Kelly Lyke³, Bryan White³, Christian Diener⁴, Osbaldo Resendis-Antonio⁵, Jaime Gransee³, Tumpa Dutta³, Xuan-Mai Persson³, Lisa Boardman³, David Larson³, Heidi Nelson³

¹Mayo Clinic Found, United States, ²Ohio State University, United States, ³Mayo Clinic, United States, ⁴National Institute of Genomic Medicine, Mexico, ⁵Coordinación de la Investigación Científica, Red de Apoyo a la Investigación, UNAM, Mexico

Multi-omic data and genome-scale microbial metabolic models have allowed us to examine microbial communities, community function, and interactions in ways that were not available to us historically. Now, one of our biggest challenges is determining how to integrate data and maximize data potential. Our study demonstrates one way in which to test a hypothesis by combining multi-omic data and community metabolic models. Specifically, we assess hydrogen sulfide production in colorectal cancer based on stool, mucosa, and tissue samples collected on and off the tumor site within the same individuals. 16S rRNA microbial community and abundance data were used to select and inform the metabolic models. We then used MICOM, an open source platform to track the metabolic flux of hydrogen sulfide through a defined microbial community that either represented on-tumor or off-tumor sample communities. We also performed targeted and untargeted metabolomics, and used the former to quantitatively evaluate our model predictions. A deeper look at the models identified several unexpected but feasible reactions, microbes, and microbial interactions involved in hydrogen sulfide production for which our 16S and metabolomic data could not account. These results will guide future *in vitro*, *in vivo*, and *in silico* tests to establish why hydrogen sulfide production is increased in tumor tissue.

Wheat bran as a driver of gut microbiota niche diversification and spatial organisation

Kim De Paepe^{*1}, Frederiek-Maarten Kerckhof¹, Joran Verspreet², Christophe M. Courtin², Kristin Verbeke², Jeroen Raes², Tom Van de Wiele¹

¹*Ghent University, Belgium*, ²*KU Leuven, Belgium*

The spatial positioning of gut microorganisms is important with respect to their functional role in the gut ecosystem. Insoluble, indigestible food residues, such as wheat bran present both a functional niche and colonisation site to microorganisms but have been poorly studied in contrast to the mucosal environment. In a series of short-term *in vitro* incubations with faecal samples derived from ten different human individuals, we have demonstrated that the wheat bran-attached microbial community reproducibly differed from the 'luminal' community in suspension. Next-generation 16S rRNA gene amplicon sequencing identified an individually dependent subset of species enriched on the wheat bran residue and this colonisation was visually confirmed using SEM and cryo-SEM microscopic evaluation. Three differentially responding individuals were selected for a time-resolved follow-up study. Unprecedented hourly sampling revealed a dynamic microbial community, characterised by a succession of bacterial taxa alternately dominating the community over a 72h timespan. The onset of wheat bran fermentation, marked by a spike in short chain fatty acid production and increased butyrate ratio, corresponded to donor-dependent proportional increases of *Bacteroides ovatus/stercoris*, *Prevotella copri*, *Coprococcus eutactus*, *Roseburia faecis*, *Clostridium xylanolyticum* and *Ruminococcus champanellensis*. These species were predominantly enriched in the bran-attached community and have the documented ability to degrade arabinoxylan or cellulose wheat bran fibres, suggesting that wheat bran colonisation and fermentation are linked. Finally, this selective enrichment of species was evidenced to be important in sustaining diversity during a one month wheat bran intervention in the semi-continuous *in vitro* Simulator of the Human Intestinal Microbial Ecosystem.

Sympatric and allopatric polymicrobial interactions alter pathogen gene essentiality

Gina Lewin^{*1}, Apollo Stacy², Marvin Whiteley¹

¹*Georgia Institute of Technology, United States*, ²*National Institute of Allergy and Infectious Diseases, National Institutes of Health, United States*

Understanding how microbes coexist within communities is a fundamental goal in microbial ecology. Here, we broadly characterize key genes that enable microbes to coexist and ask if these mechanisms are specific to interactions with sympatric microbes or universal. Using a murine abscess model, we studied interactions formed by the oral periodontal pathogen, *Aggregatibacter actinomycetemcomitans* (Aa), with each of sixteen microbes commonly found in the oral cavity and ten microbes that are not. Quantitative PCR indicated Aa persisted in the presence of all co-infecting microbes and was more abundant than in mono-

culture when co-cultured with some microbes including the periodontal pathogen *Prevotella intermedia* and the non-oral microbe *Bacillus subtilis*. To identify Aa genes necessary for coexistence, we used high-throughput mutant fitness profiling (Tn-seq). In all co-infections, Aa mutant fitness was altered relative to mono-infection, confirming that co-culture impacted Aa physiology. Differential analyses of Aa gene fitness in co-infection with oral and non-oral microbes showed that 31 genes are more important for Aa survival with oral microbes. In contrast, over 200 genes, including genes involved in nitrate reduction, cytochrome biogenesis, and central metabolism, are more important for Aa fitness during co-infection with non-oral microbes. These data indicate that in the presence of allopatric microbes, Aa requires a broader set of functions for survival and has altered metabolism. Additional characterization of these interactions is underway. These results will expand our understanding of genes important for coexistence with diverse microbes, broadly illustrating how interactions modulate gene essentiality and illuminating how communities assemble and function.

The unleashed microbiome: How antibiotics and chemotherapy shape the microbiome in patients without immune system

Jonas Schluter^{*1}, Sejal Morjaria², Bradford P. Taylor², Eric G. Pamer², Joao B. Xavier², Ying Taur²

¹Memorial Sloan Kettering Cancer Center, United States, ²MSKCC, United States

The healthy human gut microbiome comprises a complex community of microbes that is largely driven by environmental factors such as food composition, and host secretions from the innate and adaptive immune system. Here we present data from 1394 blood cancer patients undergoing hematopoietic cell therapy (HCT) at Memorial Sloan Kettering Cancer Center where these environmental factors are severely perturbed, in particular during prolonged periods of neutropenia when patients are deplete of white blood cells.

HCT patients receive frequent and diverse courses of antibiotics; prophylactically, before and during neutropenia, and in response to fever and other indications. We collected 9700 longitudinal 16s microbiota compositions in combination with over 1000 quantitative PCR measurements of almost daily samples to investigate specific effects of antibiotics and other medications on the growth dynamics of the microbiota. A diverse microbiota during critical stages of HCT, and, in particular, high abundances of obligate anaerobic species of the healthy microbiota, have been associated with improved outcomes in HCT patients, including long-term survival. We developed a Bayesian inference approach to parameterize a mechanistic model of microbial growth, and show that in particular piperacillin-tazobactam, meropenem, and metronidazole lead to large-scale losses of obligate anaerobe members of the gut microbiota

We here characterized the activity of commonly administered antibiotics *in situ*. This paves the way towards a detailed map of potential collateral damages to the microbiome, in HCT patients but likely also in patients suffering from other diseases.

Our work is a step towards more rational antibiotic stewardship.

Gut microbial potential to form acrolein and to transform heterocyclic amines is reduced in colorectal cancer patients

Clarissa Schwab*, Zhang Jianbo, Alejandro Ramirez Garcia, Hans-Joachim Ruscheweyh, Esther Wortmann, Shinichi Sunagawa, Shana J Sturla, Lacroix Christophe
ETH Zurich, Switzerland

Bacterially formed metabolites interact with dietary compounds and impact host intestinal health, however, the specific role of many metabolites still needs to be revealed.

Bacterial glycerol/diol dehydratases (GDH) metabolize glycerol to form reuterin which is a multicomponent system consisting of 3-hydroxypropanaldehyde (3-HPA), acrolein, 3-HPA dimer and its hydrate. Acrolein is the active component contributing to antimicrobial activity and the detoxification of food-derived mutagenic heterocyclic aromatic amines (HCA).

We were interested in whether the potential of acrolein formation and HCA transformation is associated with colorectal cancer status. We used an approach combining growth and bioanalytical assays coupled with metagenomic analysis to address this relationship.

Screening human fecal metagenomes, approximately 15 taxa were identified that contributed *gdh*. Using representative strains from collections, we confirmed GDH activity and HCA transformation of *Eubacterium hallii*, *Blautia obeum*, *Flavonifracter plautii* and *Lactobacillus reuteri*. We then used *ex vivo* cultivated colonic microbiota to investigate the potential to form acrolein in the presence of glycerol. Out of 6 microbiota, 4 were GDH positive, and GDH activity correlated to the abundance of *E. hallii* and *F. plautii* *gdh*. We screened fecal metagenomes of healthy (n=103) and colorectal cancer patients (n=53) for the occurrence of *gdh*. While mean gene abundance for *gdh* was similar in both groups, the proportion of taxa that we could confirm GDH and HCA transformation positive was lower in colorectal cancer patients than in healthy individuals.

Taken together, our results provide a mechanistic model for bacterial metabolite-dietary compound interactions linked to the development of colorectal cancer.

Physiological and proteogenomic studies reveal diverse pathways of anaerobic dichloromethane metabolism

Gao Chen¹, Sara Kleindienst², Manuel Iván Villalobos Solis³, Karuna Chourey³, Robert Hettich³, Orfan Shouakar-Stash⁴, Barbara Sherwood Lollar⁵, Frank Löffler^{*6}

¹University of Tennessee/Department of Civil & Environmental Engineering, United States, ²Center for Applied Geosciences, Eberhard-Karls-University Tübingen, Germany, ³Chemical Sciences Division, Oak Ridge National Laboratory, United States, ⁴Isotope Tracer Technologies Inc. (IT2), Waterloo, Canada, ⁵Department of Earth Sciences, University of Toronto, Canada, ⁶Center for Environmental Biotechnology, Department of Civil and Environmental Engineering, and Department of Microbiology, University of Tennessee, Knoxville / Biosciences Division, Oak Ridge National Laboratory, United States

Substantial amounts of dichloromethane (DCM) are produced naturally during geologic and biologic processes, with additional DCM released by anthropogenic activities. DCM is a frequent groundwater contaminant and contributes to stratospheric ozone layer depletion. While aerobic degradation of DCM has been studied in detail, knowledge of the fate of the halogenated C1 compound under anoxic conditions is limited. Cultures of *Dehalobacterium formicoaceticum* and *Candidatus* Dichloromethanomonas elyunquensis anaerobically convert DCM to acetate, formate, hydrogen, and inorganic chloride. The organisms belong to distinct genera within the *Peptococcaceae* family, and exhibit physiological differences. While axenic *D. formicoaceticum* can be maintained with DCM as the sole energy source, DCM metabolism by *Ca. Dichloromethanomonas elyunquensis* generates hydrogen, which inhibits DCM degradation. Thus, sustained DCM degradation in the latter culture requires a hydrogen-consuming partner population (i.e., DCM degradation is a strictly syntrophic process). Genomic and proteomic analyses revealed complete Wood-Ljungdahl pathways, indicating that this pathway is involved in DCM metabolism in both organisms. Interestingly, two putative reductive dehalogenases (RDases) were expressed by *Ca. Dichloromethanomonas elyunquensis*, whereas no Rdase genes were detected on the genome of *D. formicoaceticum*. Dual carbon and chlorine isotope analysis provide additional evidence for distinct DCM degradation pathways in the two organisms. These findings suggest that members of the *Peptococcaceae* employ mechanistically distinct pathways to metabolize DCM, a finding with implications for assessing and monitoring fluxes of DCM in pristine and contaminated environmental systems.

Foraging limits microbial degradation of dispersed oil droplets in the open ocean

Vicente Fernandez^{*1}, Gabriel Juarez², Roman Stocker¹

¹ETH Zurich, Switzerland, ²University of Illinois at Urbana-Champaign, United States

Droplets of water-insoluble hydrocarbons can persist in the marine water column for very long periods of time. Biodegradation of these droplets occurs through microbes interacting with the oil-water interface. As a consequence, a common argument for

using chemical dispersants to generate small oil droplets is that it increases the available surface area for bacteria, stimulating biodegradation. However, this overlooks the microscale process of bacteria encountering and attaching to oil droplets, which occurs at a rate that depends on the ambient population size of oil-degrading bacteria. Based on direct observations of oil-degrading bacteria colonizing oil droplets, we have developed a model for scaling up single droplet interactions to a cloud of spreading polydispersed droplets coupled to local ambient population dynamics. As a result of the microscale oil degradation modeling, we find that encounters between the sparse oil droplets and microbes limit the population growth of oil degrading microbes and the subsequent degradation of oil droplets in natural, constantly diluting, marine conditions. The difficulty that bacteria have in finding insoluble hydrocarbons results in an optimal droplet size for minimizing the degradation time that is larger than the size range for which droplets remain neutrally buoyant. However, in laboratory contexts it is possible for the population dynamics of the oil degrading microbes to overwhelm the encounter process, potentially accounting for the positive lab results in which chemical dispersants increasing the rate of degradation. The biodegradation of oil droplets in natural marine environments could be years longer than predicted without explicitly modeling microscale foraging.

Changes in phenotypic heterogeneity as an adaptation of fungi to environmental stress

David Foster^{*1}, Steve McGrath², Paul Dyer¹, Simon Avery¹

¹*University of Nottingham, United Kingdom*, ²*Rothamsted Research, United Kingdom*

Phenotypic heterogeneity describes variation in a given characteristic that is evident between individual cells of an isogenic population. The extent of heterogeneity in a particular phenotype may be determined by epigenetic or genetic factors, such as specific gene-promoter sequences producing noisier gene expression. A number of characteristics displaying phenotypic heterogeneity have been identified as contributing to overall fitness, therefore heterogeneity likely has a bearing on survival under temporally stable or unstable stressor concentrations. Previous evidence from our laboratory suggests that environmental stress can select for increased levels of heterogeneity in resistance (or heteroresistance) to certain stressors. Wild yeast isolates from polluted sites were found to show elevated heteroresistance to pollutants, in comparison to isolates from nearby control sites. However, the influence of environmental stability of a given stressor on selection for heterogeneity is not well understood, nor its long-term stability as an evolved trait. Yeast and filamentous fungal isolates from long-term stressed experimental sites have been studied here to provide insight. Dose-response gradients, amongst other measures of cell-cell variation, indicate that a long-term, elevated, stable level of stress may select for a decrease in heteroresistance. This suggests that the basal level of heteroresistance may in fact be intermediate, with the potential to evolve in either direction depending on the stability and predictability of the stressor dose in the environment.

Pesticide-degrading bacteria: Kings in the field, beggars in groundwater

Benjamin Horemans*¹, Springael Dirk²

¹*KU Leuven, Belgium*, ²*KU Leuven, Belgium*

In recent years, bioaugmentation was explored again as a technique to remove micropollutants such as pesticides from drinking water by applying pesticide-degrading bacteria in existing sand filters which are part of the drinking water production process. However, pesticide-degrading bacteria from agricultural soils treated with high loads of pesticides repeatedly over the years, suddenly experience a new and harsher world in sand filters where pesticide loads are continuous but extremely low. Suddenly, being able to grow on pesticides isn't a real advantage anymore.

Challenges for pesticide-degrading bacteria to invade the sand filter environment were explored by proteomics and genomics revealing signs of starvation stress and loss of catabolic enzymes needed for pesticide degradation. Starvation clearly puts pesticide-degrading bacteria in a bad position to invade the resident sand filter biofilm community well-adapted to oligotrophic conditions in sand filters. A synthetic ecological approach using sand filter bacterial isolates revealed, however, that an 'alien' pesticide-degrading bacterium relies on associations with resident bacteria in the sand filter environment to assure its survival.

The Plastisphere: A new perspective on its specificity and pathogenicity potential

Sonja Oberbeckmann*¹, Bernd Kreikemeyer², Matthias Labrenz¹

¹*Leibniz Institute for Baltic Sea Research Warnemuende, Germany*, ²*University Medical Center Rostock, Germany*

Microplastics are known to accumulate in a wide range of marine habitats, but the microbial interactions with this anthropogenically introduced substrate are not yet understood. We investigated microplastic-dependent and -independent assemblage factors along an environmental gradient, incubating polystyrene, polyethylene and wooden pellets for two weeks along the coastal Baltic Sea, the river Warnow, and in a waste water treatment plant. The developed assemblages as well as the water communities (free-living and particle-attached fractions) were investigated applying high-throughput 16S rRNA gene sequencing.

The study revealed that mainly environmental factors influence the colonization processes, and that plastic-specific bacterial assemblages are solely formed under certain conditions, such as lower nutrient concentration and higher salinity. In comparison with natural surfaces, plastic attracted significantly higher numbers of members of the genus *Erythrobacter*. This bacterial group is known for the ability to metabolize polycyclic aromatic hydrocarbons, which have been reported to abundantly accumulate on plastics. No potentially pathogenic bacteria were found to be enriched on polyethylene or polystyrene, but the abundant colonization of

microplastics in treated wastewater by certain bacteria commonly associated with antibiotic resistance suggests microplastics as potential hotspots for horizontal gene transfer. Comparisons with field samples from different marine systems support the findings of our study. Taken together, the local environment prevalently shapes the particle colonization, but microplastic-specific assemblage factors do exist. This highlights the ecological significance of specific plastic-associated microbial communities in aquatic environments and eminently in plastic accumulation zones.

***De novo* RNA-seq deciphers an unexpected metabolic link between C and N in a complex microbiome**

Yuya Sato*, Tomoyuki Hori, Hideaki Koike, Ronald R. Navarro, Atsushi Ogata, Hiroshi Habe

National Institute of Advanced Industrial Science and Technology (AIST), Japan

Metatranscriptome analysis is a powerful tool to reveal individual microbial roles in ecosystems; however, its capability and reliability are reduced by an insufficiency of reference metagenome sequences. Accordingly, the roles of minor microorganisms tend to be masked, which is problematic because “functional importance” does not always correspond to “population abundance”. Recently, *de novo* assembly-based transcriptome analysis (*de novo* RNA-seq) was developed to overcome the dependency on reference genome data size. Here, we applied *de novo* RNA-seq to decipher individual microbial functions in the complex ecosystem of activated sludge, which has been used for wastewater treatment for over a century.

Two activated sludge bioreactors were operated under the same conditions to treat heavy-oil-containing wastewater, however, the degradation performances differed between the two reactors with unknown causes. To clarify the factors shifting the reactor performances, metranscriptome profiles and physicochemical parameters of the reactors were comprehensively analyzed.

De novo RNA-seq demonstrated that denitrifiers mainly contributed to the degradation of heavy oil, which contains toxic aromatic hydrocarbons. However, no marked difference in their gene expression levels was found. Instead, nitrification activities that fueled the denitrifiers by supplying the respiratory substrate nitrate were notably high in the high-performance reactor only. Nevertheless, the relative abundance of nitrifiers was low (i.e., <0.25% of total microorganisms).

The results indicate that nitrifiers—minor microorganisms in the activated sludge—governed the heavy-oil degradation performances of the reactors, unveiling an unexpected linkage of carbon- and nitrogen-metabolisms of the complex microbiome.

Bacterial community dynamics in the urban phyllosphere

Wenke Smets*, Karen Wuyts, Wannes Van Beeck, Roeland Samson, Sarah Lebeer
University of Antwerp, Belgium

The surface of plant leaves, also termed the phyllosphere, is a unique habitat for microbes. The bacterial communities of the phyllosphere show a great diversity with specific community dynamics throughout the growing season. Despite previous claims about the phyllosphere communities being excellent models to study microbial ecology, ecological time experiments to assess natural phyllosphere communities through sequencing are lacking. In this study, we set up an experiment to investigate the effect of an urban environment and air pollution on the dynamics of phyllosphere communities.

Fourteen ivy plants (*Hedera sp.*) of the same origin were distributed in the city of Antwerp and its surroundings at locations where air pollution concentrations were monitored. During one year, the phyllosphere composition of both the distributed plants and native plants was determined using 16S rRNA gene sequencing on the Illumina MiSeq platform.

Due to the setup of the study we managed to address a range of questions on the phyllosphere communities: (i) communities of the distributed plants evolved but did not become more similar to the communities of the native plants; (ii) successional colonization of new leaves is completed within approximately 6 weeks; (iii) persistent core taxa, location dependent taxa, and transient taxa were identified; (iv) community dynamics were affected by the degree of urban land use and air pollution.

The results in this study therefore help unravel the ecological principles that apply to natural microbial communities, and how they are affected by urbanization and air pollution.

Swarming behaviour in bacteria associated with cable bacteria filaments is closely linked to electric currents

Jesper Tataru Bjerg^{*1}, Signe Brokjær¹, Ian P.G Marshall², Paula Tataru³, Casper A. Thorup¹, Markus Schmid⁴, Lars Peter Nielsen¹, Andreas Schramm¹

¹Aarhus University, Denmark, ²Aarhus University, Australia, ³Aarhus University, Romania, ⁴University of Vienna, Germany

Cable bacteria are long, filamentous bacteria which can transfer electrons over centimetre distances by coupling the half reaction of sulfide oxidation in anoxic sediment zones with oxygen reduction at the sediment surface. In freshwater sediment enrichments we observed diverse motile bacteria swarming around segments of cable bacteria in the anoxic zone. The swarming was transient, occurring only when the cable bacterium extended all the way to oxygen, several millimetres away. Cell tracking of the swarming bacteria showed that they spend most of their time within 50 µm of the cable filament and were highly diverse in their cell morphology. Amplicon sequencing of sediment enrichments selecting for motile bacteria show that chemoorganotrophic bacteria are differentially more abundant when cable bacteria are present, relative to the cable free controls. Swarming cells increase their swimming speed near the cable bacterium, and the detection frequency by fluorescence in situ hybridization is increased, indicating a higher ribosome content. This indicates that the interaction with the cable bacteria is metabolically positive to the swarming cells. The swarming ceases within one minute in cable parts that were disconnected from the oxygen exposed parts by cutting with a dissection laser microscope. Preliminary Raman microscopy of swarming cells suggests the redox state of their cytochrome c is more oxidized near the cable bacteria. These results strongly suggest some type of electron exchange whereby the swarming bacteria take advantage of long distance electron transfer by cable bacteria.

Connecting virions to their hosts by single cell viral tagging

Mária Džunková^{*1}, Soo Jen Low¹, Joshua Daly¹, Li Deng², Christian Rinke¹, Philip Hugenholtz¹

¹University of Queensland, Australia, ²Helmholtz Zentrum München - German Research Center for Environmental Health, Germany

Identification of bacteriophages' hosts is important for understanding the shift of bacterial population, or microbiome diversity spectrum, in personalized medicine. However, the modelling of such a phage-host network is complicated by the fact that no cultures (plaques) are available for most phages, and their genome sequences are generally unknown. The available computational methods for predicting uncultured phage-host pairs are essentially observational and do not attempt to study active phage infections in different environmental conditions. Therefore, we aimed to develop an experimental approach providing physical evidence for the ability of an uncultured phage to infect a range of uncultured bacterial hosts.

By sequencing 108 single bacterial cells with fluorescently labelled virions attached to their surface, we identified 210 putative phage-host pairs in faecal samples of 11 human volunteers, most of which were novel. Half of these host-phage pairs were confirmed in multiple single-cells from the same volunteers. In addition, we performed a single-cell crossover experiment in which viruses and bacteria from different volunteers were combined simulating invasion by new phages introduced during faecal microbial transplant. Bacterial composition of the viral-tagged bacteria in each cross-tagged sample was driven by the introduced phages.

The single-cell viral tagging method allows experimental confirmation of uncultured phage-host pairs and reveals more of the phage-host network than computational prediction methods. By combining viruses and bacteria from different individuals we showed that phages from one individual are able to infect bacterial hosts from other individuals, which may have implications for faecal microbiota transplants.

Microalgal farming in planktonic symbiosis through structural and metabolic transformation

Decelle Johan^{*1}, Hryhoriy Stryhanyuk², Benoit Gallet³, Giulia Veronesi⁴, Clarisse Uwizeye⁴, Giovanni Finazzi⁴, Yannick Schwab⁵, Nicole Schieber⁵, Niculina Musat²
¹LPCV Grenoble-CNRS, France, ²Helmholtz Center for Environmental Research UFZ, Germany, ³Institut de Biologie Structurale (IBS, CEA, CNRS), France, ⁴CEA/CNRS, France, ⁵EMBL, Germany

Photosymbiosis between single-celled hosts and microalgae is widely distributed in the oceanic plankton. However, the functioning of this ecologically-important interaction remains enigmatic, particularly the mechanisms that allow the host to exploit its intracellular microalgae. Here, using a combination of quantitative single-cell structural (3D electron microscopy) and chemical imaging (nanoSIMS, synchrotron X-ray fluorescence), we show how the host reconfigures the photosynthetic machinery and metabolism of its microalgae. Within the host, photosynthetic efficiency is enhanced and the volume of algal cells increases up to ten times with a higher number of chloroplasts and thylakoid membranes, compared to the free-living stage (outside the host). Subcellular mapping of nutrients and N/P ratios shows that symbiotic microalgae are limited in phosphorous (more particularly in their chloroplasts), and are transformed into an energy-acquisition machinery (i.e. higher N/P ratio compared to the host and the free-living stage). In addition to N, the host also supplies a substantial amount of essential metals (iron and cobalt) that are stored in high concentration in algal vacuoles, very likely to sustain the high primary productivity of its symbionts. Overall, this study sheds the light on specific mechanisms of a host to engineer microalgae in the oceanic plankton and brings new insights into chloroplast acquisition in eukaryotes.

Flow of methane-derived carbon through a microbial community in a stratified lake

Sina Schorn^{*1}, Jon Graf¹, Sten Littmann¹, Philipp Hach¹, Gaute Lavik¹, Marcel M. M. Kuypers¹, Carsten Schubert², Jana Milucka¹

¹Max Planck Institute for Marine Microbiology, Germany, ²Swiss Federal Institute of Aquatic Science and Technology (Eawag), Switzerland

Methane-oxidizing bacteria (i.e. methanotrophs) have the unique capacity to use methane as a carbon and energy source and thereby form the basis of a methane-driven food web. Methanotrophs are often found physically associated with other non-methanotrophic bacteria, such as methylo- and heterotrophs. It is speculated that these bacteria feed off carbon compounds that are formed during methane oxidation and methane assimilation; thus, their growth is indirectly supported by methane.

Here we investigated the flow of methane-derived carbon through a microbial community in a stratified lake using stable isotope labeling and nanoscale secondary ion mass spectrometry (nanoSIMS). We collected samples from the permanently stratified Lake Zug (Switzerland) that is characterized by an anoxic, methane-rich (> 30 μM) hypolimnion. Incubations with ^{13}C -labeled methane revealed high rates of $^{13}\text{CO}_2$ production (up to 2 $\mu\text{M day}^{-1}$) under both oxic and anoxic (denitrifying) conditions. Using catalyzed reporter deposition fluorescence *in situ* hybridization we identified active methanotrophs belonging to the *Gammaproteobacteria* (gamma-MOB) in all investigated water depths. NanoSIMS analyses revealed increasing ^{13}C enrichment in the biomass of individual gamma-MOB over time. Interestingly, various bacteria associated with the gamma-MOB also became enriched over time, indicating that they grew on methane-derived carbon compounds. Our results highlight the importance of methane-derived carbon for microbial communities in stratified lakes and demonstrate the power of single-cell approaches for studying microbial interactions in the environment.

Illuminating microbial growth controlling network in nature

Eun-Young Seo^{*1}, Yuki Takagi², Tomonori Kindaichi², Akiyoshi Ohashi², Yoshiteru Aoi¹

¹Department of Molecular Biotechnology, Hiroshima University, Japan, ²Department of civil and environmental Engineering, Hiroshima University, Japan

Significance of microbial interactions in nature is not well understood, due to limitations of technique to observe growth activity at single cell level under the condition similar to that in nature. We hypothesized that most of the microbes in natural environment are in non-growing state (dormant) and require inter- or intra-species interactions for triggering growth (resuscitation). The aim of the study is to reveal microbial growth controlling network in nature. We established a new microbial cultivation platform providing extremely high inoculum cell density (>10⁷ cells/ml) but keep individuality of each growth unit (single cells and micro-colonies). Hydro-gel particles (10-30 μm in diameter) entrapping single cells with medium softly

aggregated in oil (GMD-oil cultivation) is the key structure of this method. As a result of comparative experiment on colony formation using soil and activated sludge sample, the cultivation efficiency showed approximately 10 times higher than the condition without microbial interactions. This suggested that the enhancing microbial interactions facilitate microbial resuscitation from dormancy.

Next, selected isolates from above method were individually tested on recovery from dormancy under the same condition (GMD-oil cultivation) but co-cultured with other isolates (different species) or environmental samples. Cultivation efficiencies of tested isolates were significantly higher when co-cultured with specific isolates or environmental sample (mixed species) than pure-cultures. Accordingly, inter-species microbial interactions are significant factor for growth triggering in a microbial community. This suggests that microbial network controls growth of specific microbial type in nature, and it might be a major reason for microbial unculturability.

Transparent soil microcosms to visualize soil microbial activity and decomposition at the microscale

Kriti Sharma^{*1}, Marton Palatinszky², David Berry², Elizabeth A Shank¹

¹University of North Carolina, United States, ²University of Vienna, Austria

Carbon cycling in the soil depends on the micrometer scale processes of microbial extracellular enzyme production and uptake of decomposition products. However, due to the complexity of soils, there is a dearth of tools available to understand microbial community activity and decomposition at this scale. We have established a multi-modal imaging platform to non-destructively visualize microbial activity in soil-like environments and to map the movement of carbon in soil communities at the micrometer and millimeter scales in three dimensions over time. These “transparent soil microcosms” consist of 5 to 50 μm particles of Nafion (an inert polymer that is transparent when hydrated) in microfluidic chambers. Our model system consists of the fungus *Mucor fragilis* and the soil bacterium *Bacillus subtilis*, engineered with a fluorescent reporter for the *csn* gene encoding an extracellular chitosanase. By growing fungal spores within a microcosm with ^{13}C -glucose and heat-killing the fungus *in situ*, we provide *B. subtilis* with a complex, isotopically labeled sole carbon source. We can then quickly and non-destructively measure uptake of this ^{13}C -labeled polysaccharide by *B. subtilis* cells within the Nafion matrix using Raman microspectroscopy. In addition, by integrating fluorescence microscopy, we can simultaneously monitor chitosanase expression levels using the *B. subtilis* fluorescent *csn* reporter. This approach allows us to monitor – at the single cell level, in a spatially resolved way and over time – which cells produce extracellular enzymes, and which cells take up the products of decomposition.

Soil-on-a-Chip: Exploring microbe-microbe interactions using microfluidic technology

Claire Stanley
Agroscope, Switzerland

Soil is one of the most complex systems on Earth, governed by numerous physical, geochemical and biological processes, and provides the ecosystem services vital for terrestrial life. This 'material' supports a myriad of plants, microorganisms and microfauna and hosts a complex array of interactions taking place between these living elements. However, despite the importance of microbes in soil functioning, there exists a major knowledge gap concerning the function and dynamics of the soil microbiome and influence of the physio-chemical environment upon microbial interaction and communication at the cellular level.

Recently, it has been demonstrated that microfluidic technology can offer new opportunities to study whole living organisms and their interactions and has a great potential to provide a unique view of biological events at the level of single organisms and cells (i.e. microbe–microbe interactions). I have developed microfluidic systems to probe interactions between fungi, bacteria and nematodes, which has revealed novel insights into the antagonistic strategies of these microorganisms including bacteria-induced blebbing of hyphal cells, as well as discovering that undifferentiated mycelium can communicate within certain microdomains using previously unknown specialised hyphae. The dual-flow-RootChip allows investigations on the interaction of plant roots with their environment under simulated environmental heterogeneity, revealing that external asymmetry can result in internal asymmetry at the physiological and genetic level. We are now developing new microfluidic tools to investigate microbial interactions in the rhizosphere, specifically to probe the cell biology and physiology of microbe association and colonisation of arbuscular mycorrhizal fungi at the cellular level.

Stability in diversity: Numerous functionally distinct symbiont strains coexist in deep-sea bivalve symbioses

Rebecca Ansorge^{*1}, Stefano Romano², Lizbeth Sayavedra³, Anne Kupczok⁴, Tal Dagan⁴, Halina Tegetmeyer³, Nicole Dubilier³, Jillian Petersen²

¹Max Planck Institute for Marine Microbiology, Germany, ²Department for Microbiology and Ecosystem Science, University of Vienna, Austria, Austria, ³Max Planck Institute for Marine Microbiology, Bremen, Germany, Germany, ⁴Christian-Albrechts-University Kiel, Germany, Germany

Microbial strain variability is virtually universal, but disentangling the genomes of closely-related strains from complex communities typically found in nature remains challenging. Symbiotic deep-sea mussels are ideal for understanding the functional and evolutionary consequences of strain variation due to the natural simplicity of their symbiont communities: Each mussel has one highly abundant 16S rRNA phylotype of symbiotic sulfur-oxidizing bacteria, hosted inside gill epithelial cells. We sequenced metagenomes from 18 symbiont-hosting mussels collected from their entire known distribution range in the Atlantic. Despite their identity at the 16S rRNA level, we found that up to 16 distinct symbiont strains coexist in individual hosts. Although symbiont heterogeneity within host individuals was high, population genetic analyses revealed that co-occurring mussels shared similar symbiont populations and possibly exchange symbionts throughout their lifetime. Up to 50% of all symbiont genes were strain-specific, encoded only by some but not all co-occurring strains. These covered a surprisingly wide range of functions including key metabolic pathways, gene regulation, and interacting with hosts or viruses. Most strain-specific genes were expressed, highlighting their functional and adaptive potential. Current evolutionary theory predicts that extensive strain diversity in symbiont populations is unstable over evolutionary time, even though it provides major selective advantages for adapting to changing environments and evading viruses. We have shown that strain diversity is pervasive in the *Bathymodiolus* symbioses. We hypothesize that hosts can maintain stable relationships with multiple symbiotic partners, particularly in such 'low-cost' chemosynthetic symbioses where the symbionts are fed by the environment rather than the host.

A link to the past: newly recovered Asgard genomes reinforce the archaeal eukaryotic ancestry

Paul-Adrian Bulzu^{*1}, Adrian-Stefan Andrei², Rohit Ghai², Maliheh Mehrshad², Horia Banciu³

¹Babes-Bolyai University, Romania, ²Institute of Hydrobiology CAS, Department of Aquatic Microbial Ecology, Czech Republic, ³Babes-Bolyai University, Faculty of Biology and Geology, Department of Molecular Biology and Biotechnology, Romania

In light of recent research, eukaryotes emerged within the archaeal domain of life, where they share close evolutionary ancestry with the enigmatic Asgard superphylum, a group of uncultivated archaea comprising the Loki-, Thor-, Heimdall- and Odinarchaeota. Originally identified in deep sea vents, recently some have been recovered from brackish environments. Despite the fact that this clade

paved the way to one of the most innovative transitions in genome evolution, it currently suffers from lack of phylogenetic resolution and metabolic characterization. Here, we use genome-resolved metagenomics to reconstruct and characterize Asgardarchaeota genomes present in two sediment samples from South-Eastern Romanian brackish lakes. By using *de novo* assembly and taxonomy-dependent and -independent binning we recovered nine nearly-complete and 26 partial genomes that effectively doubled the amount of available genomic data. Phylogenomic analysis not only placed our genomes within the Asgard superphylum, it also reiterated the intriguing topology that depicts Eukarya branching from within. Furthermore, we expanded the known Asgard repertoire of membrane remodelling and intracellular vesicular transport components (mostly absent in all other archaea) by performing sensitive searches against eukaryotic protein databases. Our genome-scale metabolic inferences showed that the anaerobic lifestyles of Loki- and Thorarchaeota lineages were replaced in Heimdallarchaeota by an aerobic one, setting the latter in the metabolic vicinity of the eukaryotic counterparts. Although, at present, the eukaryotic ancestor is still elusive, our results corroborate previous findings regarding the topological backbone of the tree of life and provide new compelling metabolic information in support of the Asgardarchaeota-Eukarya ancestry.

Specialization and diversification in nectar-dwelling bacteria: Genome evolution associated with growth in floral nectar and pollinator dispersal

Tory Hendry^{*1}, Rachel Vannette²

¹Cornell University, United States, ²University of California, Davis, United States

How and why some bacteria become specialized to certain hosts or habitats remains poorly understood, but is fundamentally important to the ecological and evolutionary dynamics of microbiome communities. Many of the microbes that dwell in floral nectar are highly specialized for the challenges of the environment, such as very high sugar levels and plant defense compounds. These microbes may also rely on floral visitors for dispersal. However, some of these taxa, such as *Acinetobacter* species, have close relatives that are generalists in a variety of habitats. We used this system to explore the genomic changes and evolutionary processes that are associated with habitat specialization. We performed genomic and molecular evolutionary analyses on the *de novo* sequenced genomes of 9 strains of nectar-specialist bacteria compared to the genomes of close relatives that have more generalist habitat ranges. Nectar specialist clades have experienced increased evolutionary rates compared to relatives and gene loss consistent with habitat restriction. These genomes also contain a number of habitat specific genes, most of which are associated with plasmids or mobile genetic elements. Despite having slightly reduced genomes compared to relatives, nectar specialists have very high numbers of mobile genetic elements, including plasmids and prophages, and this may be associated with diversification between lineages. We also discuss potential connections between strain specific genes, mobile elements, and association of different species with specific floral visitors. Overall, these data suggest that both habitat driven gene loss and horizontal gene transfer are important for specialization in nectar-dwelling bacteria.

Clade-specific patterns of reductive genome evolution in ancient thiotrophic endosymbionts

Oliver Jäckle*, Brandon K. B. Seah, Nicole Dubilier, Harald R. Gruber-Vodicka
Max Planck Institute for Marine Microbiology, Germany

The marine flatworm *Paracatenula* lacks a mouth and gut, and gains its nutrition from chemosynthetic alphaproteobacteria named *Ca. Riegeria*. These intracellular symbionts are strictly vertically transmitted, which is known to lead to rampant genome reduction. However, given the complete reliance of the flatworm on its symbionts for its nutrition, there should be strong selective constraints on symbiont genome reduction.

To trace how reduced genomes evolved in *Ca. Riegeria*, we sequenced 35 symbiont genomes of 23 *Paracatenula* species from the Mediterranean and the Caribbean. The symbiont genomes had sizes between 1.19 to 2.04 Mb and GC contents of 45.1 to 55.2%. Gene losses that affected parts of the DNA repair and cell division machinery were specific for some *Ca. Riegeria* lineages. This lineage-specific gene loss appeared to be stochastic, and ranged from nucleotide-centered erosions to full gene deletions. Genome synteny was lineage-specific and highly variable, with one *Ca. Riegeria* lineage displaying several 100-fold more rearrangements than other lineages of similar age. Despite the central importance of autotrophic carbon fixation in the symbioses, one of the key metabolic genes for carbon fixation, *cbbL*, has seen replacement through horizontal gene transfer. Our analyses revealed that current models of genome evolution of vertically transmitted endosymbionts, which are largely based on insect-bacteria symbioses, do not adequately represent the stochastic nature of the process or the strong stabilizing selection observed in the *Paracatenula* – *Ca. Riegeria* symbiosis.

Microorganisms putatively involved in nutrient recycling of ant-plant associations by degrading chitin and cellulose

Maximilian Nepel*¹, Andreas Richter², Christina Kaiser², Dagmar Woebken¹,
Veronika Mayer³

¹*Division of Microbial Ecology, Department of Microbiology and Ecosystem Science, University of Vienna, Austria,* ²*Division of Terrestrial Ecosystem Research, Department of Microbiology and Ecosystem Science, University of Vienna, Austria,* ³*Division of Structural and Functional Botany, Department of Botany and Biodiversity Research, University of Vienna, Austria*

Many canopy-inhabiting (arboreal) ants feed mainly on carbohydrate-rich but nitrogen-poor hemipteran exudates or plant-derived food, and thus need to overcome nutrient limitations. Until now it has been assumed that some arboreal ant species harbor endosymbionts that are involved in synthesis of amino acids and

other organic acids, or feed on Chaetothyriales (Ascomycota) fungi to satisfy their nutrient demand.

In the myrmecophytic *Azteca* ssp. (Dolichoderinae) – *Cecropia* ssp. (Urticaceae) association, the plant is providing shelter and nutrient-rich food bodies in exchange for protection against herbivores. Inside the nest the ants maintain patches build from parenchyma and dead nest mates that also harbor fungi and bacteria. It was the goal of this study to investigate whether microorganisms inhabiting these patches could be directly involved in the ant's nutrient provision or recycling.

By applying stable isotope-labeled substrates to patches, we could demonstrate that patch components represent a nutrient source for *Azteca* larvae during colony foundation. Patch microbial biomass seemed to be supplied and maintained by cellulose and chitin degradation, and investigations are ongoing targeting the fungal or bacterial participants. Amplicon sequencing of the 16S rRNA genes of patch biomass revealed a high bacterial diversity, including potential cellulose- and chitin-degrading OTUs belonging to e.g. Chitinophagaceae, Sphingomonadaceae, or Rhizobiaceae. Another aim of the investigation is to reveal the transfer mechanism of patch nutrients to the ant larvae.

We hypothesize that this multi-species network, in which fungi and bacteria degrade polymers and thus allow a recycling of nutrients, is essential to maintain ant colonies in the canopy.

Prophages are responsible for the presence of Zonula occludens toxin in bacterial pathogens that infect different hosts

Esther Rubio-Portillo^{*1}, Ana G Cobián-Güemes², Cynthia B. Silveira², Josefa Antón³, Forest Rohwer²

¹Univeristy of Alicante, Spain, ²San Diego State University, United States, ³University of Alicante, Spain

Phages can contribute to bacterial pathogenicity by horizontal transfer of virulence genes by a phenomenon known as lysogenic conversion. *Vibrio cholerae* represents a paradigm for this process, the major virulence factors of this pathogen are encoded by the filamentous prophage CTX. One of these virulence factors is the zonula occludens toxin (Zot), which regulates intercellular tight junctions in human epithelial cells. Here we show that the Zot gene is encoded by prophages in distinct bacterial species that infect human and scleractinian corals, eukaryotic hosts at extremes of phylogenetic distance. Filamentous prophages related to CTX encode the Zot toxin in the coral pathogens *Vibrio coralliilyticus* and *Vibrio mediterranei* isolated from bleached corals, and the gene is over-expressed at high temperature. Likewise, we detected the Zot gene in a metagenomic study of human lungs during an episode of cystic fibrosis exacerbation. Zot was encoded by *Stenotrophomonas maltophilia* prophage SHP1. Meta-analysis of Zot-encoding prophages demonstrates the presence of this virulence factor in the plant pathogens *Xanthomonas campestris* and *Xanthomonas axonopodis*, which is encoded by prophages with a 59% of similarity with *Stenotrophomonas* phages. It is generally

assumed that phages have a narrow host range and the genetic exchanges are restricted to closely-related strains. Our work shows that prophages could be involved in the spread of Zot toxin among phylogenetically distinct bacterial species infecting highly divergent eukaryotic hosts.

Cultivated relationships: genetics of microbiome programming of host physiology

Fan Zhang¹, Jessica Weckhorst¹, Marie-Anne Felix², Buck Samuel^{*1}

¹Baylor College of Medicine, United States, ²École Normale Supérieure (ENS), Institut de biologie de l'ENS (IBENS), France

Partnerships between animals and microbes are ancient and born out of mutual benefit. Proper establishment and maintenance of these relationships relies on communication. To understand the host (and microbial) signaling pathways that regulate these processes, we employ the genetically tractable, high-throughput amenable nematode *Caenorhabditis elegans*. Its natural microbiome is acquired horizontally and deterministically, plus exerts immense influence on host physiology.

To define these genetic programs, we first established a model microbiome [68-members, fully sequenced genomes]. Natural variation in colonization and microbiome composition was then monitored [42 previously 'germ-free' wild *C. elegans* strains] using a high-throughput gut colonization method that we developed and 16S sequencing. We observed a 30-fold range in colonization levels with the lab N2 strain and 4 others among the 'poor controllers'. Deterministic selection was observed for most strains tested (~75%), though six 'poor selectors' lack these programs as gut microbiomes resembled the lawn. No strains were defective in both selection and control, suggesting compensatory genetic programs for microbiome regulation.

We then identified genes involved in microbiome control (512) and selection (499) using GWAS and machine-learning methodologies. These candidates classify into novel and highly conserved pathways common in other systems for microbiome regulation—e.g., GPCRs, mucus, regulation of metabolism, gut motility and immunity. A prioritized RNAi sub-library (364 genes) were screened and validated for their role in microbiome control and colonization defects. Together, these studies further our understanding of how the microbiome programs host physiology and development in this simple host.

Illuminating dark matter diversity in forest soil: A mini-metagenomic approach

Lauren Alteio^{*1}, Frederik Schulz², Alexander Truchon¹, Aria Jordan¹, Rex Malmstrom², Tanja Woyke³, Jeffrey Blanchard¹

¹*University of Massachusetts Amherst, United States*, ²*DOE Joint Genome Institute, United States*, ³*DOE Joint Genome Institute, United States*

Forest soils harbor nearly two-thirds of global carbon stores supporting an immense diversity of microorganisms, of which nearly 99% remain uncultivated. Bulk metagenomics has been used as a cultivation-independent approach to characterize the vast diversity of soil organisms, however it is dependent on curated databases and genome assembly from soil remains challenging. Here we apply mini-metagenomic sequencing of microorganisms from soils collected at the Harvard Forest long-term warming experiment to reveal hidden biodiversity and develop more complete reference genomes for hypothesis-driven research. We extracted and stained viable cells using SYBR green, followed by Fluorescence Activated Cell Sorting and sequencing of 90 pools of 100 cells. This mini-metagenomic approach resulted in ~2,000 genome bins, and generated longer contigs with nearly 20x more contigs assembling than in a bulk metagenome from the same soil sample. Phylogenomic analysis revealed previously unknown giant viruses with genome and particle sizes larger than many bacteria, as well as novel Chlamydiae genomes sequenced independently from any eukaryotic host cell. Additionally, mini-metagenomics revealed an astonishing degree of diversity within novel groups across taxonomic levels. Many of these taxa are observed in soils globally and have relevance to food webs and biogeochemical cycles, but have only been surveyed using rRNA and have few isolated representatives. Comparative and pangenomic analyses of the genome bins will further reveal ecologically-relevant traits. Reference genomes generated through mini-metagenomics are being used to map bulk metagenomic and metatranscriptomic reads to more closely link organisms to functions within this soil community under conditions of climatic shift.

Recovery of near closed genomes of member species from enrichment reactor microbial communities using long read metagenomics

Irina Bessarab¹, Krithika Arumugam¹, Xianghui Liu¹, Anna Thi Quynh Ngoc Nguyen¹, Gayathri Natarajan¹, Guanglei Qiu¹, Stefan Wuertz¹, Federico Lauro², Yingyu Law¹, Daniel Huson³, Rohan Williams^{*1}

¹*Singapore Centre for Environmental Life Sciences Engineering, Singapore*, ²*Nanyang Technological University, Singapore*, ³*University of Tubingen, Germany*

New long read sequencing technologies offer a huge potential for effective recovery of complete, closed genomes. While much progress has been made on cultured isolates, the ability of these methods to recover genomes of member taxa in complex microbial communities is less clear. Here we examine the ability of long read data to recover genomes from enrichment reactor metagenomes. Such modified communities offer a moderate level of complexity compared to the source

communities and so are a realistic, yet tractable, system to use for this problem. We sampled a series of enrichment bioreactors designed to target anaerobic ammonium-oxidising bacteria (AnAOB) or glycogen accumulating organisms (GAO), extracting genomic DNA and obtaining both short read (Illumina 251 bp or 301 bp PE) and long read data (MinION Mk1B) from the same DNA aliquot. In the AnAOB reactor, the community had 17 members having relative abundance over 1%, with the most abundant member being an AnAOB (24%). Assembly of short read data recovered 4 complete genome bins (completeness > 95%, contamination <5%; 45-1520 contigs) on a background of 16 detected genomes, which included two distinct AnAOB species. Assembly of the long read data recovered a 3.5 Mb genome (<3 contigs) that is attributable to the most abundant AnAOB species obtained from the short read assembly. Similarly, from the GAO reactor we obtained a comparable draft genome for *Candidatus Competibacter*. Long read metagenomics in enrichment bioreactor communities of medium complexity is feasible and can recover near-closed draft genomes of abundant community members.

Candidatus Prosiliococcus vernus*, gen. nov., sp. nov., a spring phytoplankton bloom associated member of the *Flavobacteriaceae

Ben Francis*, Karen Krüger, Bernhard Fuchs, Hanno Teeling, Rudolf Amann
Max Planck Institute for Marine Microbiology, Germany

Microbial degradation of algal biomass following spring phytoplankton blooms has been characterised as a concerted effort among multiple clades of heterotrophic bacteria. Many of these clades have so far resisted cultivation. One clade known from 16S rRNA gene sequencing of surface waters at the island of Helgoland in the North Sea was formerly identified as belonging to the genus *Ulvibacter*. This clade rapidly responds to algal blooms and can transiently make up as much as 20% of free-living bacterioplankton. Sequence similarity of less than 95% between the 16S rRNA genes of described *Ulvibacter* species and those detected at Helgoland indicate that this clade is a distinct novel genus. This is supported by phylogenetic analyses based on 40 metagenome assembled genomes derived from samples collected during spring blooms at Helgoland during the years 2010-2012. These represent three species within the genus, only one of which appears to truly bloom in response to the phytoplankton. The most complete genomes from the two lower abundant species ranged between 45-65% and 76-91%, while the most abundant species produced a genome that was 92-96% complete. Functional predictions based on genome sequences indicate this highly abundant species most likely consumes a mix of proteins and the abundant diatom derived polysaccharide laminarin. Fluorescence in situ hybridisation indicated cells are coccoid, with a diameter of approximately 500-1000 nm (mean: 781 nm). We here propose the novel candidate genus *Candidatus Prosiliobacter*, and for the most abundant and well characterised of the three species the name *Candidatus Prosiliobacter vernus*.

Discovery and functional description of novel gammaproteobacterial diversity in sponges

Viet Hung Nguyen^{*1}, Torsten Thomas¹, Nicole Webster², Holly Bennett³, Bernd Wemheuer¹, Weizhi Song¹

¹University of New South Wales, Australia, ²Australian Institute of Marine Science, Australia, ³Victoria University of Wellington, Australia

Sponges are ubiquitous in the world's oceans and are known to harbor diverse and complex microbial communities. Recent global surveys have shown that the microbiomes of most sponge species are dominated by Gammaproteobacteria. While this class has many well-characterised taxa and has been extensively studied, many of the sponge-associated 16S rRNA gene sequences found could not be classified below the class level. This indicates that sponges carry novel, unexplored diversity within the Gammaproteobacteria.

Here we use 30 microbial metagenomes of the sponges *Stylissa flabelliformis* and *Carteriospongia foliascens* to reconstruct 30 genomes representing eight novel gammaproteobacterial species, which surprisingly represent seven novel orders based on 16S rRNA gene sequences and average amino acid identities to reference genomes. The species were predominantly found in sponges and hence likely represent sponge-enriched/specific symbionts.

Functional analysis predicts metabolic differentiation across the species. While all are capable of chemoorganoheterotrophic growth, they can do so under aerobic (3 species), anaerobic (2 species) or both aerobic and anaerobic (3 species) conditions. Four species are capable of metabolizing sponge-derived compounds, with two of them being particularly versatile in their use of organic compounds. Two species are capable of thiosulfate oxidation, while another two are capable of reducing nitric oxide to nitrous oxide. These differentiations might be important to allow for the occupation of different niches in the sponge environment.

Our study has thus revealed previously "hidden" taxonomic and functional diversity within the Gammaproteobacteria and has expanded the previous ~14 orders in the class by another 7 novel orders.

Expanding archaeal diversity from deep-sea hydrothermal systems

Anna-Louise Reysenbach^{*}, Emily St John, Jennifer Meneghin
Portland State University, United States

Actively venting deep-sea hydrothermal deposits (chimneys) are quickly colonized by a rich diversity of thermophilic Archaea. It was shown that the structure of the archaeal communities is shaped by geochemical and physical conditions of the sulfide deposits. While these findings transformed our understanding of linkages between archaeal community composition and geologic setting, they highlighted

how much of the archaeal tree remains underexplored. Most notable was the unprecedented abundance and diversity of Crenarchaeota and the presence of conspicuously understudied archaeal lineages such as the Korarchaeota, Nanoarchaeota, and Aigarchaeota. Analysis of 10 metagenomes from samples collected from several vents fields along the Eastern Lau Spreading Center in the southwestern Pacific, resulted in about 2,300 draft metagenome assembled genomes (MAGs), of which 349 (over 92 >70% complete, <10% contamination) were archaeal. In addition to the increased diversity of genomes from the Thermococcales, Archaeoglobales and Desulfurococcales, several MAGs of lineages rarely or never before reported from deep-sea vents, were characterized and classified using average amino acid identity (AAI) and average nucleotide identity parameters (ANI). These included relatives of *Caldisphaera*, *Vulcanisaeta*, *Ignisphaera*, and members in the Nanoarchaeota, Korarchaeota, Aigarchaeota, Asgaard and the Nanohaloarchaeota; many represent new genera and families. Some of the common metabolisms among the lineages were anaerobic heterotrophy and CO oxidation.

MiGA—The Microbial Genomes Atlas: Expanding the catalogued diversity of *Archaea* and *Bacteria* and getting quantitative insights into the species issue

Luis M Rodriguez-R^{*1}, Gunturu Santosh², Jain Chirag¹, Ramon Rosselló-Mora³, James Tiedje², James Cole², Konstantinos T Konstantinidis¹

¹Georgia Institute of Technology, United States, ²Michigan State University, United States, ³Universitat de les Illes Balears, Spain

The use of 16S rRNA gene sequencing has enabled the large-scale cataloguing of prokaryotic species but offers limited resolution at genus or species levels and cannot assess functional gene diversity. These limitations have been circumvented with advances on (meta)genomics techniques but the exploration of newly uncovered diversity in geometrically increasing prokaryotic genomes remains computationally challenging. Here, we present the Microbial Genomes Atlas (MiGA), which essentially represents the genome equivalent of the rRNA web servers, integrating best practices in genomic analyses with novel developments in taxonomy and classification. MiGA features an indexing system based on medoid clustering over matrices of Average Nucleotide and Amino Acid Identity (ANI/AAI), enabling the fast classification of query sequences against all classified reference genomes. The recent development of FastANI, a k-mer-based ANI estimator integrated in MiGA, allowed us to explore the distribution of ANI values among >90 thousand complete and draft genomes. This distribution revealed a pronounced valley in the ANI range 83-95%, indicating that the genetic discontinuity previously observed with smaller sets of genomes, reflective of discrete species, is maintained in larger scales regardless of taxonomic diversity or historic sequencing trends. The availability of this indexed set of genomes, as well as metagenome-assembled genomes from various large-scale projects and curated collections like RefSeq and RefSoil allows the rapid search for close relatives of any complete or draft query genomes including uncultivated and not-yet-classified genomes. MiGA has an easy-to-use web interface available through <http://microbial-genomes.org/>.

Cable bacteria: from single filament taxonomy to clonal enrichments in autoclaved sediment

Casper Thorup*¹, Jesper Bjerg¹, Caitlin Petro², Andreas Bøggild³, Signe Nielsen¹, Lars P. Nielsen¹, Andreas Schramm¹

¹*Center for Electromicrobiology, Department of Bioscience, Aarhus*

University, Denmark, ²*Section for Microbiology, Department of Bioscience, Aarhus*

University, Denmark, ³*Department of Molecular Biology and Genetics, Aarhus University, Denmark*

Cable bacteria are multicellular, centimeter-long filamentous bacteria that conduct electrons from sulfide in deeper sediment layers to oxygen at the sediment surface. This recently discovered life form, based on long-distance electron transfer, is functionally and morphologically conspicuous but has so far evaded isolation into pure culture. Sequencing of partial 16S rRNAs combined with fluorescence in situ hybridization (FISH) initially placed cable bacteria within the *Desulfobulbaceae* family. Whole genome amplification (WGA) using individually picked cable bacteria filaments resulted in almost complete 16S rRNA and dissimilatory sulfite reductase (*dsrAB*) genes, which formed the basis for proposing two novel candidate genera, the marine *Ca. Electrothrix* and the freshwater *Ca. Electronema*. However, cable bacteria genomes obtained by WGA are incomplete and fragmented, resulting in erroneous genome-to-genome comparisons and uncertain taxonomic assignments. The alternative to WGA, assembly of cable bacteria genomes from metagenomes, is hampered by high cable bacteria (micro)diversity in natural sediments. Here we present a method to establish clonal cable bacteria enrichments, facilitating assembly of high-quality cable bacteria genomes for taxonomy and omics analyses.

Autoclaved freshwater sediment was inoculated with single cable bacteria filaments using glass hooks. FISH and microsensor measurements confirmed the development of active cable bacteria. After repeated single-filament transfers, an almost complete genome of a new cable bacterium species could be reconstructed from a mini-metagenome. Comparing average nucleotide identities between transfers verified the clonal nature of the cable bacterium enrichment. Similar approaches with single-cell transfers into natural medium may aid the genomic characterization and taxonomic delineation of other hard-to-culture microbes.

Genome-resolved metagenomics illuminates evolutionary links between methanogenesis, anaerobic methanotrophy, and short-chain alkane oxidation

Guillaume Borrel*, Panagiotis Adam, Simonetta Gribaldo
Institut Pasteur, France

Over the past few years, our long-term vision of methanogenesis has been profoundly modified by the discovery of novel methanogen lineages distantly related to all methanogens previously known. A striking characteristic of these new archaea is their limitation to a previously unusual type of methanogenesis (the reduction of methyl-compounds with H₂), associated with the lack of several enzymes classically involved in methanogenesis. Moreover, it has been recently discovered that some archaeal lineages possess a novel enzymatic complex, sharing similarities with the MCR complex responsible for the production of methane in all methanogens. This MCR-like complex is involved in a new pathway of short-chain alkanes oxidation.

By mining thousands of available metagenomes, we have reconstructed ten archaeal genomes corresponding to new lineages of predicted methyl-dependent hydrogenotrophic methanogens, methanotrophs and short-chain alkane oxidizers. The large distribution of the methane-metabolisms in the Archaea supports the recent hypothesis that the last common ancestor of the domain was a methanogen. Members of one of these new lineages are the first to possess both a MCR-like complex along with a classical MCR enzyme, which may be used in a novel methanogenesis pathway based on short-chain alkanes. We defined a core of marker genes shared only by methanogens, methanotrophs and short-chain alkane oxidizers that tightens the functional and evolutionary relationships between these metabolisms. Our results reveal possible evolutionary links between these metabolisms that could represent intermediate steps toward the loss of methanogenesis and the diversification of metabolisms/lifestyles that occurred during the evolution of Archaea.

Exploring the metabolic versatility of "Candidatus Nitrosocaldus islandicus," an obligately thermophilic, ammonia-oxidizing Thaumarchaeon

Anne Daebeler*¹, Craig W Herbold¹, Anna Müller¹, Julia Vierheilig¹, Christopher J Sedlacek¹, Petra Pjevac¹, Mads Albersten², Rasmus H Kirkegaard², Jose R de la Torre³, Holger Daims¹, Michael Wagner¹

¹University of Vienna, Austria, ²Aalborg University, Denmark, ³San Francisco State University, United States

Ammonia-oxidizing archaea (AOA) within the phylum Thaumarchaeota are key nitrifiers in many ecosystems including geothermal environments. Although molecular data indicate the presence of phylogenetically diverse AOA in terrestrial high-temperature habitats, only very few enrichment cultures (and no pure culture) of AOA thriving above 50°C have been reported and functionally analyzed. From a hot spring in Iceland we have enriched (85% of all cells) and characterized the obligately thermophilic strain "Candidatus Nitrosocaldus islandicus" belonging to the Nitrosocaldales, which grows at 50 to 75°C by chemolithoautotrophic ammonia oxidation. Comparative genomics revealed many unexpected features such as the

absence of a *nirK* gene and other known enzymes for nitric oxide (NO) generation. Nevertheless, ammonia oxidation by this AOA appears to be NO-dependent as it is inhibited by the addition of an NO scavenger. Therefore, this AOA may produce NO via a yet unidentified mechanism or rely on NO produced by other microbes.

Interestingly, we also discovered the potential for facilitated fermentation of aromatic amino acids by "Ca. N. islandicus". Its genome encodes key enzymes needed for this pathway, an indolepyruvate oxidoreductase (*iorAB*) and a type 3b hydrogenase. To confirm this first nitrification-independent lifestyle found in AOA we have incubated "Ca. N. islandicus" under anaerobic conditions, assessed growth and transcriptional activity, and monitored substrate consumption and product formation. Attempts to isolate the AOA under fermentative conditions are ongoing. Results of these physiological experiments will be presented and put into context of the ecophysiology and evolution of ammonia monooxygenase-encoding Thaumarchaeota.

Atmospheric trace gas oxidation: a novel primary production strategy in oligotrophic ecosystems

Chris Greening^{*1}, Mukan Ji², Sean Bay³, Angelique Ray², Carlo Carere⁴, Inka Vanwonderghem⁵, Kiel Hards⁶, Ralf Conrad⁷, Osnat Gillor⁸, Zahra Islam³, Joanna Feng³, Philip Hugenholtz⁵, Michael Berney⁹, Matthew Stott⁴, Gregory Cook¹⁰, Belinda Ferrari²

¹Monash University, Australia, ²School of Biotechnology and Biomolecular Sciences, University of New South Wales, Australia, ³School of Biological Sciences, Monash University, Australia, ⁴GNS Science, New Zealand, ⁵Australian Centre for Ecogenomics, University of Queensland, Australia, ⁶Department of Microbiology and Immunology, University of Otago, New Zealand, ⁷Max-Planck Institute for Terrestrial Microbiology, Germany, ⁸Department of Environmental Hydrology and Microbiology, Ben Gurion University of the Negev, Israel, ⁹Department of Microbiology and Immunology, University of Otago, Australia, ¹⁰Department of Microbiology and Immunology, University of Otago, Dunedin, Otago, New Zealand

Most microbial cells exist within a spectrum of dormant states with their environment. The metabolic processes that enable them to survive environmental challenges, such as nutrient limitation, remain to be resolved. Here we provide evidence that the atmospheric trace gases molecular hydrogen (H₂) and carbon monoxide (CO) serve as alternative energy sources for aerobic bacteria. Genetic and biochemical studies show that axenic mycobacterial cultures use these gases as respiratory electron donors when exhausted for preferred organic carbon sources. This alternative metabolic pathway is tightly regulated, critical for redox homeostasis and necessary for long-term survival. The determinants of this process are widespread in the genomes of aerobic bacteria and we have experimentally validated that four dominant bacterial phyla scavenge these gases. Biogeochemical and metagenomic profiles show that trace gas scavenging is highly active at the ecosystem level. Atmospheric trace gases are particularly important energy sources for bacterial communities in oligotrophic ecosystems and we provide evidence that they are major energy sources supporting primary production in the surface soils of hyper-arid deserts. These

findings provide new understanding of the the biogeochemical hydrogen cycle and the minimal nutritional requirements for life.

Carbon metabolism of versatile anaerobic ammonium-oxidizing bacteria resolved by isotope labeling and systems-level metabolic flux analysis

Christopher Lawson^{*1}, Tyler Jacobson¹, Joshua Hamilton¹, Rob de Graaf², Guylaine Nuijten², Mike Jetten², Daniel Noguera¹, Katherine McMahon¹, Daniel Amador-Noguez¹, Sebastian Lücker²

¹University of Wisconsin-Madison, United States, ²Radboud University, Netherlands

Anaerobic ammonium-oxidizing (anammox) bacteria play a central role in global nitrogen cycling and mediate energy-efficient wastewater treatment processes for autotrophic nitrogen removal. While significant progress has been made on understanding the energy metabolism of anammox bacteria, limited physiological studies have been done to confirm their central metabolism beyond genomic predictions. Here, we employ a systems biology approach to resolve the metabolic network of the anammox bacterium *Candidatus Kuenenia stuttgartiensis* using stable isotopes combined with quantitative metabolomics and metabolic network modeling. ¹³C-labeled bicarbonate was fed to planktonic cultures of *Candidatus Kuenenia stuttgartiensis* growing in membrane bioreactors, followed by rapid metabolite quenching and extraction over a time course of three hours. Samples were analyzed via high-resolution liquid chromatography mass spectrometry (LC-MS) to follow ¹³C incorporation into the metabolome. Metabolite labeling patterns were used to resolve the metabolic network structure of *Ca. K. stuttgartiensis* and compute intracellular fluxes via isotopic non-stationary metabolic flux analysis. In-vivo fluxes and absolute metabolite levels were used to calculate pathway thermodynamics, and were combined with transcriptomic data to refine a genome-scale metabolic reconstruction of *Ca. K. stuttgartiensis*. Our findings reveal novel deviations in the central metabolism of anammox bacteria from what was predicted based on genomics alone and represents a platform for incorporating quantitative systems biology into environmental biotechnology and biogeochemistry.

Uncultured Bathyarchaeota in termite guts are novel archaeal homoacetogens

Hui Qi Loh^{*}, Vincent Hervé, Andreas Brune
Max Planck Institute for Terrestrial Microbiology, Germany

Symbiotic digestion of lignocellulose in the hindgut of higher termites is mediated by a diverse assemblage of bacteria and archaea. An important intermediate in the fermentative breakdown of polysaccharides is hydrogen, which drives both the methanogenic and acetogenic reduction of CO₂. While methanogenesis has been attributed to hydrogenotrophic Euryarchaeota colonizing the different gut compartments, the identity of the microorganisms responsible for the homoacetogenic activities remains unclear. In the course of a large-scale metagenomic study of the

gut microbiota of several higher termites, we reconstructed the genomes of 38 uncultured Archaea. Based on phylogenomic analysis, a large proportion of these genomes formed a distinct clade in the radiation of the recently proposed phylum Bathyarchaeota (formerly Miscellaneous Crenarchaeota Group), which represent a large proportion of the archaeal community in higher termites. Comparative analysis of the genomes indicate that the uncultured Bathyarchaeota in termite guts are obligate anaerobes that possess the complete pathway for reductive acetogenesis from H_2 and CO_2 , using tetrahydromethanopterin as the C_1 carrier. Marker genes for methanogenesis were absent. Our results suggest that Bathyarchaeota contribute to homoacetogenesis in the guts of higher termites and add to the genomic evidence for hydrogen-dependent CO_2 reduction in other uncultured representatives of the phylum.

***Smithella propionica* LYP uses a novel fourth pathway for syntrophic propionate degradation**

Masaru Konishi Nobu^{*1}, Catalina Hidalgo², Takashi Narihiro¹, Wen-Tso Liu³, Alfons Stams², Neil Wofford⁴, Michael McInerney⁴, Diana Sousa²

¹National Institute of Advanced Industrial Science and Technology (AIST), Japan, ²Wageningen University & Research, Netherlands, ³University of Illinois, Urbana-Champaign, United States, ⁴University of Oklahoma, United States

Propionate is a common product of odd-chain fatty acid, amino acid, and sugar decomposition and can be toxic at high concentrations. To prevent propionate accumulation and toxicity, all domains of life encode propionate-catabolizing pathways. So far, three pathways have been characterized (*i.e.*, methylmalonyl-CoA pathway, acrylyl-CoA pathway, and 2-methylcitrate cycle), but de Bok *et al.* (2001) showed that *Smithella propionica* LYP employs an uncharacterized fourth pathway. Although stable isotope analyses revealed that two propionate molecules are condensed and oxidatively split into three acetate molecules, the detailed biochemistry of this unique pathway remained unclear. To elucidate the gene systems involved, we sequenced LYP's genome and performed transcriptome analysis on a propionate-degrading co-culture of LYP with *Methanobacterium formicicum*. By combining gene expression, protein phylogeny, and protein modeling, we successfully identified the genes and enzymes responsible for the unusual transformation of propionate to acetate. Notably, LYP uses a novel 2-methyl-3-oxo-valeryl-CoA mutase and 4-hydroxycaproyl-CoA dehydratase phylogenetically and likely functionally unique from any known homologs. In methanogenic environments where *Smithella* inhabits, the conditions are thermodynamically restricting and propionate decomposition requires "syntrophic" electron transfer between a propionate oxidizer and electron-receiving partner. Inspection of LYP's genome reveals an unusual set of enzymes for transferring reducing power from propionate oxidation to H^+ and CO_2 respiration. For example, LYP did not encode conventional energy conservation (*e.g.*, electron-confurcating hydrogenase) that other syntrophic organisms encode. The results suggested that the novel propionate degradation pathway is mechanistically and energetically distinct from known pathways, which has significant ecological implications for syntrophy in methanogenic ecosystems.

Enrichment and physiological characterization of a novel comammox *Nitrospira*

Dimitra Sakoula*, Maartje AHJ van Kessel, Jeroen Frank, Laura N Wenzel, Theo A van Alen, Mike SM Jetten, Sebastian Lückner
Radboud University, Netherlands

Nitrification, the oxidation of ammonia to nitrate via nitrite, was considered to be catalyzed by two functionally distinct groups of microorganisms. However, the recent discovery of complete ammonia oxidation (comammox) by members of the genus *Nitrospira* overturned this paradigm. The widespread occurrence of comammox bacteria in natural and engineered ecosystems indicates their important role in the biogeochemical nitrogen cycle. In order to study their physiology and environmental niche adaptation, highly enriched cultures are required. Here, we enriched a comammox *Nitrospira* from a recirculating aquaculture system using a continuous membrane bioreactor, supplied with limiting concentrations of ammonium and oxygen. Metagenomic analysis and quantitative fluorescence *in situ* hybridization indicated that comammox *Nitrospira* were the only ammonia-oxidizing organisms present and constituted approximately 80% of the microbial community. Additionally, high-throughput combined with long-read sequencing yielded the closed genome of a novel comammox *Nitrospira* species. Physiological characterization of the enrichment culture demonstrated high affinities for both ammonium and oxygen. The calculated ammonia affinity was in the range of non-marine ammonia-oxidizing archaea, but slightly below that of *N. inopinata*. Surprisingly, strong substrate inhibition was observed at extremely low ammonium concentrations. These data indicate the adaptation of the enriched comammox *Nitrospira* to highly oligotrophic environments, as was predicted to be the main environmental driver selecting for comammox bacteria. In conclusion, this study presents novel physiological aspects for the first comammox enriched from an engineered habitat and further expands our understanding of the ecological niche of these organisms in the environment.

A tale of two spaces: unravelling the mechanisms by which intracellular and gut symbionts provide nutritional benefits to their animal hosts

Nana Ankrah*, Angela Douglas
Cornell University, United States

Many animals of medical and agricultural importance depend on symbiotic associations with nutrient producing bacteria. Very commonly, net nutrient production by the bacteria is dictated by metabolic competition and cooperation among multiple bacterial taxa and strongly influenced by the metabolites derived from the animal host, but the mechanistic details of the multi-way metabolic interactions are poorly understood. To investigate the processes shaping animal-microbe metabolic interactions and to derive estimates of the composition and amount of nutrients exchanged between animal and microbe, we reconstructed genome and transcriptome-informed metabolic models of sap-feeding insects that harbor intracellular bacterial partners and the bacterial community in the *Drosophila* gut. Our simulations reveal that among intracellular bacterial partners the insect host controls bacterial production of nutrients, by precise controls over the concentrations of substrate metabolites in essential nutrient biosynthetic pathways. The net result is the structuring of the metabolic networks of the bacteria, to promote cooperative cross-feeding of metabolites and minimize competition for host-derived substrates. Among gut microbes competitive interactions dominate and the quality and quantity of nutrients available to the host is influenced by the composition of gut microbiota which synthesize and consume host and microbe derived nutrients. Our studies elucidate the metabolic basis of animal-microbe interactions and provide the methodology to identify specific gene targets for novel pest control strategies and to select the optimal microbial consortia for personalized microbial therapies in treating human diseases associated with microbial dysbiosis.

Mutualism and parasitism amongst coral symbioses in the midst of global change

David Baker
The University of Hong Kong, Hong Kong

Coral reefs are the most biodiverse ecosystems on Earth. Their success through deep time is afforded through a multi-Kingdom symbiosis with microbial partners. The most important of these are endosymbiotic *Symbiodinium*, a hyper-diverse genus of dinoflagellates which confer an autotrophic metabolism to the coral meta-organism. However, coral reefs are suffering catastrophic losses owing to the synergistic effects of ocean warming and eutrophication, causing coral bleaching. Our understanding of this process is limited by a widespread belief that *Symbiodinium* are obligate mutualists that only serve to supply the host with energy, when in reality they are fierce competitors for the host habitat and must therefore have the capacity to sequester resources for growth and reproduction; at

times, at the expense of their host. As such, *Symbiodinium* can function along a spectrum from mutualist to parasite.

Here, I will show how the "strength" of the coral symbiosis can now be quantified using stable isotope measurements of the host and symbiont, and further resolved through controlled thermal stress experiments. Bayesian ellipse analysis of the isotopic "niche" reaffirms long-held hypotheses that large-polyped corals are more heterotrophic and resistant to coral-bleaching. Moreover, high-temperature isotope tracer experiments revealed that *Symbiodinium* metabolism is enhanced with warming, resulting in increased carbon and nitrogen assimilation and mitotic index while hosts incur a heavy cost from respiration. Taken together, these studies provide a mechanistic explanation for predictions of "winners" and "losers" on coral reefs due to climate change, and demonstrate that *Symbiodinium* are unlikely to save corals from extinction.

Evolution of the chlamydial symbiotic lifestyle: insights from the discovery of novel and diverse lineages from deep marine sediments

Jennah E. Dharamshi^{*1}, Courtney Stairs¹, Joran Martijn¹, Felix Homa¹, Laura Eme¹, Steffen L. Jørgensen², Anja Spang³, Thijs J. G. Ettema¹

¹Uppsala University, Sweden, ²University of Bergen, Norway, ³NIOZ Royal Netherlands Institute for Sea Research, Netherlands

Known Chlamydiae share an obligate symbiotic lifestyle with eukaryotic hosts, despite forming an ancient phylum-level clade. We identified chlamydial rRNA in a metagenome from sediment near Loki's Castle, a deep-sea hydrothermal vent field on the Arctic-Mid-Ocean-Ridge. Given the evolutionary relevance of chlamydia in symbiosis, pathogenesis and the evolution of plastids, the finding warranted further investigation. Bacterial 16S rRNA amplicon sequencing of 30 deep marine sediment samples from near Loki's Castle revealed unprecedented chlamydial abundances and taxonomic diversity with relative chlamydial abundance approaching 43% of the total bacterial population, and up to 163 chlamydial OTUs, in individual samples. Phylogenetically these amplicons both span and expand known chlamydial diversity. Chlamydiae were found at various depths throughout several sediment cores, indicating their prevalence under diverse ecological conditions. Using a metagenomic approach we have reconstructed 24 novel chlamydial genomes from these marine sediments, with high completeness. Nearly doubling sequenced chlamydial species, phylogenetically these lineages compose both novel and deep-branching clades. Analysis of gene content from these novel genomes redefines the genomic diversity within Chlamydiae, and includes the identification of anaerobic metabolism within the phylum, with genes involved in hydrogen production that are related to eukaryotic homologs. Such findings question our current understanding of chlamydial biology, and suggest that some Chlamydiae members could be having an impact on marine biogeochemistry. This expanded chlamydial diversity has revealed novel insights into the evolution of obligate symbiosis and pathogenesis in Chlamydiae, and also provided new clues about their role in major evolutionary transitions.

Vampire Vibrios: Persistent, internal associations with blood-feeding marine invertebrates

Shana Goffredi^{*1}, Ralph Appy², Rebecca Hildreth¹, Julia DeRogatis¹

¹Occidental College, United States, ²Cabrillo Marine Aquarium, United States

Nearly all blood-feeding animals examined so far, from tsetse flies to vampire bats, host internal bacterial symbionts that aid in some aspect of their nutrition. Similarly hematophagous invertebrates exist in the oceans, yet symbiotic associations between them and beneficial bacteria have not been explored. This study describes the prevalence of a single bacterial genus, *Vibrio*, within 10 phylogenetically-diverse species of marine 'vampires', including leeches (both fish and elasmobranch specialists; ex. *Pterobdella* and *Branchellion*, resp.), isopods (ex. *Elthusa* and *Nerocilla*), copepods (ex. *Lernanthropus*), and nematodes (ex. *Philometra*). In each case, the specific *Vibrio* OTU, and its abundance were unique compared to other invertebrates and biological surfaces. In some cases, *Vibrio* was observed, based on sequencing of the 16S rRNA gene, also associated with eggs and developing hatchlings. In the fish leech *Pterobdella*, bacteria were localized to nephridia along the body wall, a phenomenon also seen in freshwater leeches. In the shark leech *Branchellion*, bacteria were found only in the crop in and amongst the blood meal. In other hosts, *Vibrio* cells were obvious, but have not yet been localized to a specific tissue, based on fluorescence and TEM microscopy. Cultivation of 5 of these *Vibrio* species revealed their ability to digest red blood cells and recycle urea, perhaps hinting at a nutritional role. Virtually nothing is known about the influence, if any, of internal bacteria on the success of marine blood-feeders, and this study provides evidence for their presence in several prominent marine parasites.

Mutualist co-evolution in natural microbial 'Pink Berry' consortia: a population genomics perspective

Gabriel Leventhal^{*1}, Jakob Russel², Rachel Soble², Tim Enke², Jose Saavedra², Otto Cordero², Boris Shraiman³, Elizabeth Wilbanks⁴

¹Massachusetts Institute of Technology (MIT), United States, ²Massachusetts Institute of Technology, United States, ³KITP, UC Santa Barbara, United States, ⁴UC Santa Barbara, United States

Microbial populations generally display a large amount of genomic variation across space and time, and understanding how this variation relates to adaptations to biotic and abiotic environmental changes is one of the fundamental goals of microbial ecology and evolution. Many natural bacterial populations, however, exist as members of larger communities, in which they are often intricately linked to other members in symbiotic relationships. To what degree these interactions within communities are lineage-specific and, hence, shaped by co-evolution is much less known.

Here, we make use of a naturally occurring consortium—the pink berries of Sippewissett—and perform a population genomic analysis of ~150 individual consortia. The consortia were collected with a nested geographical pattern: from 3 large-scale sites (km-scale), multiple ponds within each site (100m-scale), and multiple berries from within each pond (m-scale). The nested geographical pattern allows for an unprecedented analysis of the evolutionary history of the two dominant member species: a purple sulfur bacterium (PSB; *Thiohalocapsa*) and a sulfate-reducing bacterium (SRB; *Desulfofustis*). These two populations are intimately linked in a syntrophic sulfur cycling relationship. We find that there is a remarkable amount of genomic variation on the SNP-level across populations from individual berries and that the evolutionary history of the PSB recapitulates that of the SRB. We then investigate specific genomic regions of PSB and SRB to uncover candidates that show signatures of co-evolution between the PSB and SRB populations. Our genomic analysis of the PSB-SRB symbiosis provides a unique opportunity to investigate the coevolution of natural bacterial populations.

Comparative analysis of host-associated microbiomes: phyllosymbiosis, co-diversification and co-evolution

Florent Mazel^{*1}, Mathieu Groussin², Laura Parfrey¹

¹University of British Columbia, Canada, ²Massachusetts Institute of Technology, United States

Host-associated microbiota composition varies dramatically between host species. Intriguingly, closely related hosts often harbor similar microbiota. The measurement of the prevalence of this phylogenetic *pattern*, sometimes referred to as “phyllosymbiosis” have gained much attention recently. However, the mechanisms at play behind this pattern remain controversial. In particular, is phyllosymbiosis the result of co-evolution? Here, we first review the literature and measure the prevalence of phyllosymbiosis in the real world. Second, we show, using simulations, how simple ecological filtering – without any co-evolutionary dynamics— can produce weak, but detectable, phyllosymbiosis patterns. However, these simulations do not recover the strong and widespread signal found in the guts of animals. Using real data, we show that co-diversification of mammals and their gut microbes lead to strong phyllosymbiosis. Intriguingly, highly co-diversifying bacterial genera are also associated with immune diseases in humans, laying a path for future studies that probe these co-diversifying bacteria for signs of co-evolution.

(Meta)genome sequencing of the *Porites lutea* holobiont, a common reef building coral, illuminates the roles of critical microbial symbionts

Steven Robbins^{*1}, Cheong Xin Chan², Hua Ying³, Francesco Rubino⁴, Sara Bell⁵, Kathleen Morrow⁶, David Miller⁷, Christian Voolstra⁸, David Bourne⁹, Gene Tyson¹

¹Australian Centre for Ecogenomics, University of Queensland, Australia, ²Institute for Molecular Biosciences, University of Queensland, Australia, ³Australian National

University, Australia, ⁴Australian Center for Ecogenomics, University of Queensland, Australia, ⁵Australian Institute for Marine Sciences, Australia, ⁶George Mason University, United States, ⁷Department of Environmental Science and Policy, James Cook University, Australia, ⁸King Abdullah University of Science and Technology, Saudi Arabia, ⁹Australian Institute of Marine Science, Australia

Although microbial communities play a critical role in the provisioning and cycling of nutrients within the coral holobiont, the nature of their interactions with the coral host remain poorly understood. To address this gap, the ReFuGe2020 consortium has generated genomic data for the complete community of organisms that constitute healthy reef-building corals to clarify their individual roles in maintaining coral health. This effort has permitted, for the first time, all of the major components of the holobiont to be sequenced and characterized, including the coral host and its microbiome. Here, we present an integrated analysis of the genomes of the Indo-Pacific reef building coral, *Porites lutea*, its dominant *Symbiodinium* strain (clade C15), and 52 bacterial and archaeal genomes. These data represent the first genome-resolved investigation of coral-associated microbial communities and have major implications for understanding the metabolic interactions between members of the coral holobiont. Several bacterial and archaeal lineages, some also dominant in surveys of marine sponges, demonstrated the genomic potential to supply fixed carbon, b-vitamins, and amino acids to the eukaryotic components of the holobiont, as well as participate in the cycling of sulfur and nitrogen. Additionally, we identify specific gene-sequence motifs across the bacterial and archaeal genomes that could facilitate establishment and maintenance of association with the coral host. This research provides a baseline for understanding the role of the microbial communities that contribute to coral health and the potential for adaptation in a changing marine environment.

Microbial carbon utilization in forest topsoil: Feed in summer, rest in winter

Petr Baldrian

Institute of Microbiology of the CAS, Czech Republic

Evergreen coniferous forests contain high stocks of organic matter. Significant carbon transformations occur in litter and soil of these ecosystems, making them important for the global carbon cycle. Due to seasonal allocation of photosynthates to roots, carbon availability changes seasonally in the topsoil. Seasonal differences in C source utilization and the involvement of various members of soil microbiome in this process were studied in forest topsoil. Microorganisms in topsoil encode a diverse set of carbohydrate-active enzymes, including glycoside hydrolases and auxiliary enzymes. In each enzyme family, hundreds to thousands of genes are typically transcribed simultaneously indicating high functional redundancy of forest soils and the involvement of thousands of microbial taxa. Transcripts of genes encoding enzymes targeting plant biomass biopolymers, reserve compounds and fungal cell walls are especially abundant in the coniferous forest topsoil. While the transcription of genes encoding enzymes degrading reserve compounds, such as starch or trehalose, is high in soil in winter, summer is characterized by high transcription of ligninolytic and cellulolytic enzymes produced mainly by fungi. Fungi strongly dominate the transcription in litter while the contribution of bacteria and fungi to transcription in soil is equal. The turnover of fungal biomass appears to be faster in summer than in winter, due to high activity of enzymes targeting its degradation, indicating fast growth in both litter and soil. Seasonal differences in the transcription of glycoside hydrolases and auxiliary enzyme genes are more pronounced in soil than in litter due to the changing allocation of photosynthate-derived C into soil.

Unlinked rRNA operons are common in soil bacteria

Tess Brewer*, Noah Fierer

University of Colorado Boulder, United States

When a protein is crucial to the function of a cell, its components are usually highly resistant to change and evolutionarily constrained. For example, textbooks teach that the RNA components of the ribosome (rRNA) are organized into a single operon conserved across all prokaryotic life. This arrangement allows all rRNA to be transcribed, processed, and regulated together - a feature thought to be important for fast growth.

In reality, there are some prokaryotes that do not share this canonical rRNA order; their 16S and 23S rRNA are separated and referred to as "unlinked". Culture independent studies have recently revealed that many free-living, environmental prokaryotes have unlinked rRNA operons. These prokaryotes are under selection to minimize detrimental mutations to their genomes and even possess genetic machinery capable of correcting rogue genome rearrangements - suggesting unlinked rRNA operons may offer an evolutionary advantage.

Unlinked rRNA operons are relatively common in soil - roughly 25% of prokaryotic rRNA in some soils is unlinked, a much higher fraction than in other environments (~0.5% in the human gut). Additionally, certain ubiquitous and abundant soil bacteria (*Verrucomicrobia* of the class *Spartobacteria*) also possess unlinked rRNA operons. These two facts imply that unlinked operons may be especially advantageous in soil. This talk will focus on exploring which taxa have unlinked rRNA operons using novel techniques and synthetic long read sequencing technology, along with what the potential evolutionarily advantage of this arrangement may be.

How phage and fauna shape the flow and fate of root carbon through microbial pathways

Mary Firestone^{*1}, Javier Ceja-Navarro², Evan Starr¹, Henrik Krehenwinkle¹, Shengjing Shi³, Angela Hodge⁴, Rosemary Gillespie¹, Eoin Brodie², Jizhong Zhou⁵, Jillian Banfield¹, Jennifer Pett-Ridge⁶

¹University of California, United States, ²Lawrence Berkeley National Laboratory, United States, ³AgResearch Ltd, New Zealand, ⁴University of York, United Kingdom, ⁵University of Oklahoma, United States, ⁶Lawrence Livermore National Laboratory, United States

Stimulated by exudates and decaying roots, rhizosphere organisms interact to move carbon from roots into soil and ultimately influence soil carbon persistence. This project addresses three classes of interactions: arbuscular mycorrhizal fungi, bacteria, and roots; phage and their microbial hosts; and rhizosphere fauna (proto- and metazoan) and their prey.

¹³CO₂-grown plants introduce carbon into soil to delineate the complex web of biotic interactions. Isotope-enabled genomics and genome-resolved metagenomics reveal phage, bacterial, fungal, and faunal participants; food-web and network modeling identify and quantify guilds contributing to soil C persistence. *Avena fatua*, (common in Mediterranean grasslands) was grown under ¹³CO₂ and rhizosphere and bulk soil collected over time. Extracted DNA underwent density-gradient centrifugation, yielding discreet ranges of ¹³C label; DNA was then sequenced, data were assembled, binned for host genomes, and phage contigs.

Populations of phages that incorporated label into their genomes differed from non-labelled phages, indicating that the development of rhizosphere-competent bacterial consortia enabled production of different phage populations. Several phages were linked with hosts; phage-host abundances differed between samples. Rhizosphere phages infected root-carbon-dependent bacteria.

The physical separation of nematodes and protists from soil involved gradient centrifugation and size selection by differential filtration. Subsamples of isolated nematodes and protists underwent DNA extraction, metabarcoding, fixing, and imaging. Sequenced libraries yielded 50-70% faunal sequences. Nematode and protist densities were thus assessed in bulk and rhizosphere. Predicted functional roles of these guilds suggest direct root feeding and bacterial and fungal predation. These functional predictions are being evaluated using isotopic methods.

Microbial activities and fluxes of greenhouses gases in high-arctic tundra soils

Aline Frossard^{*1}, Vivien Hotter², Ramona Kern², Lotte DeMaeyer³, Bjorn Tytgat³, Ulf Karsten², Mette Svenning⁴, Elie Verleyen³, Beat Frey⁵

¹*Swiss Federal Institute for Forest, Snow and Landscape Research (WSL), Switzerland*, ²*University of Rostock, Germany*, ³*Ghent University, Belgium*, ⁴*UiT The Arctic University of Norway, Norway*, ⁵*Swiss Federal Research Institute WSL, Switzerland*

High-arctic tundra-soil ecosystems are particularly sensible to global changes due to their proximity to freezing, snow cover, light availability and scarcity of vegetation. Hydrological fluctuations in these soils are also important, where drier soils are expected to be less buffered to temperature variation, directly impacting soil biological activity. Yet, little is known on the effects of soil moisture in the regulation of microbial activities in high-arctic soils and their impact on greenhouses gas exchanges with the atmosphere. The fluxes of greenhouse gases (CO₂, CH₄ and N₂O) were assessed along a moisture gradient (30 - 70 %) in high-arctic tundra soils near Ny-Ålesund (Svalbard) and linked to microbial extracellular enzyme activities and functional genes. Fluxes of CO₂ were highest in dry soils, indicating higher respiration compared to wetter soils in which CO₂ fixation was more important than respiration. Methane (CH₄) was mostly consumed except for the wettest soils where CH₄ was emitted. Carbon acquiring enzyme activities decreased with increasing moisture in tundra soils. Similarly, C:N and C:P enzymatic ratios were higher in the drier soils. These results highlight the importance of soil moisture in high-arctic tundra soils for microbial activities and their impacts on carbon cycling and greenhouse gases fluxes.

Circumpolar Arctic microbial communities respond to warming and vegetation changes

Sara Hallin^{*}, Jaanis Juhanson, Mathilde Jeanbille, Karina Clemmensen
Swedish University of Agricultural Sciences, Sweden

The arctic tundra is experiencing changes due to global warming causing greening of the Arctic. However, studies of how soil microbial communities are affected are lacking, which hampers general conclusions concerning warming effects on tundra ecosystems. We analyzed the bacterial, fungal and nitrogen cycling communities in soil from 19 warming experiments, each with 4 control plots and 4 open-top chambers, within the International Tundra Experiment (ITEX) across the Arctic. We sequenced the fungal ITS region and bacterial 16S rRNA gene and quantified N-cycling genes. Warming had a negative effect on mosses and lichens, and a positive effect on shrubs in some sites, indicating that warming effects are site specific. Although these warming-induced changes were not reflected in the below-ground microbial community composition, the microbial co-occurrence networks were modified by warming. The fungal network was reorganized by warming with

increased path length and a modification of the modules. Modules, indicating fungi with sheared niche, were related to sites and vegetation, suggesting a site-dependent response to experimental warming. For bacteria, the modules were preserved between control and warming, with similar relationships with soil nutrients and N-cycling genes. However, a turnover of putative keystone species was observed between conditions. Also, the negative co-occurrences were denser in the warming plots, which is indicative of disturbance as they typically increase stability of complex networks under perturbation. In conclusion, warming resulted in disturbances on potential interactions within the microbial communities, which could impact nutrient cycling and above- and belowground interactions.

Complete denitrification at pH 4 in peat circles of the arctic Tundra is primarily driven by acetate assimilating *Burkholderiaceae*

Stefanie Hetz*, Marcus A. Horn
University of Hannover, Germany

The assembly of a functional nitrous oxide (N₂O) reductase of classical neutrophilic model denitrifiers is impaired at pH < 6 resulting in increased N₂O/N₂ (dinitrogen gas) ratios. Accordingly, acidic peat circles (pH 4) in the Eastern European Tundra, with up to 2 mM pore water nitrate, emit the greenhouse gas N₂O like heavily fertilized agricultural soils in temperate regions. The main process yielding N₂O under anoxic conditions is denitrification, i.e. the sequential reduction of nitrate to N₂O and N₂. Organic carbon to nitrate ratios and pH are crucial factors impacting denitrification and N₂O/N ratios. Active key denitrifiers of peat circles are important but hitherto unknown. Thus, it is hypothesized that acid tolerant peat circle denitrifiers are new, impaired by pH and unable to reduce N₂O. Anoxic microcosms +/- supplemental nitrate and +/- acetylene (N₂O reductase inhibitor) at *in situ* pH 4 were used to test the effect of [¹³C]- and [¹²C]-acetate on denitrification and N₂O production. Relative to unsupplemented controls, nitrate alone stimulated N₂O production by 1000 % and supplemental acetate with nitrate stimulated N₂O production by 330 %, with rather than without acetylene, suggesting complete denitrification at pH 4. *Burkholderiaceae*, other Proteo-, and Actinobacteria as well as Verrucomicrobia, were identified as key acetate assimilating denitrifiers in peat circles via 16S rRNA SIP. Collective data indicate that peat circles host new complete denitrifiers capable of N₂O reduction at pH 4 that operate under substrate limitation in peat circles and thus produce large amounts of nitrate derived N₂O.

Uncovering microbial metabolic interactions in thawing permafrost degradation via mixed meta-omics

Robert Hoelzle*¹, Ben Woodcroft², Malak Tfaily³, Gene Tyson²

¹*Australian Centre for Ecogenomics, The University of Queensland, Australia*, ²*Australian Centre for Ecogenomics, School of Chemistry and Molecular Biosciences, The University of Queensland, Australia*, ³*Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, United States*

An estimated 174Pg of organic carbon stored as permafrost will thaw by the year 2100, becoming available for microbial degradation and contributing to atmospheric greenhouse gas accumulation. The quantities of the key greenhouse gasses CO₂ and methane that will be produced from thawing permafrost and the metabolic pathways involved in degradation are currently unknown, but are critical to predicting the contribution atmospheric greenhouse levels. In this study, we incubated thawed permafrost soil from Stordalen Mire (Northern Sweden) in a controlled bioreactor and linked metagenomic and metabolomic techniques to characterize the microbial pathways and interactions involved in the breakdown of organic matter. The community enriched predominantly to lineages of organic matter degraders *Paludibacter* and *Acidobacteriaceae*, and methanogen *Methanobacterium*. Curiously, the abundance profile of *Paludibacter* strongly correlated ($R^2=0.78$) with the 1-propanol production profile, though no related type-strains are known to metabolize 1-propanol or its precursors. Genome recovery and annotation revealed a complete pathway through the methylglyoxal bypass to key 1-propanol precursor 1,2-propanediol in this lineage, with a possible metabolic hand-off to a lineage of *Holophaga* for further reduction to 1-propanol. These pathways serve as effective electron sinks, drawing electrons away from potential methane production. Further analysis aims to describe additional pathways and interactions in this system, including those acting on high molecular weight plant organic matter, which was measured to decrease in average mass from 406Da to 390Da through the incubation. This analysis demonstrates the potential of mixed meta-omic analyses to uncover microbial metabolic interactions in complex ecosystems.

Viruses in soil: Nano-scale undead drivers of microbial life, biogeochemical turnover and ecosystem functions

Yakov Kuzyakov^{*1}, Kyle Mason-Jones²

¹RUDN University, Russia, ²University of Göttingen, Germany

Viruses are ubiquitous in any environment and have various ecological functions. The importance of viruses for soil life remains completely disregarded. With this review we focus on the functioning of viruses in soil and the consequences for microbial life and cycling (death and growth), as well as related mechanisms of biogeochemical cycling of carbon (C) and nutrients. Considering that the vast majority of soil bacteria are infected, and the fast lysis of infected cells, we discussed five concepts: *Viral Shunt*, 'Forever Young', *Viral Regulatory Gate of EXOMET*, *C sequestration by microbial necromass stabilization*, and *Microscale divergence of C/N/P stoichiometry*.

The '*Viral Shunt*' raises the importance of viruses for bacterial life and shows that viruses explain bacterial death and the release of easily available C and nutrients, consequently accelerating biogeochemical cycles in soil – roles previously hypothesized for the *Microbial Loop*. The concept 'Forever Young' shows that viral infection maintains the active bacterial population in soil at a young age. The two previously suggested but unexplained concepts of '*Regulatory Gate*' and '*EXOMET*' were joined based on viral cell lysis and endoenzyme release. Preferential allocation

of P to new phages compared to C and N leads to a *Stoichiometric C/N/P Imbalance* at the microscale that may have consequences for P limitation at macroscales. We discuss the concept of '*C sequestration by necromass stabilization*' from the new perspective of viral lysis. Concluding, functioning very fast at the nano-scale, the undead drivers govern microbial life in soil and biogeochemical turnover at micro and ecosystem scales.

Legacy of drying-rewetting affects the soil microbial response to freeze-thawing

Annelein Meisner^{*1}, Aimee T.classen², Joseph Nesme³, Anders Prieme³

¹Lund University, Sweden, ²Rubenstein School of Environment and Natural Resources/ University of Vermont,, United States, ³University of Copenhagen, Denmark

Terrestrial ecosystems will experience altered frequency and intensity of drying-rewetting and freeze-thaw events due to climate change. These fluctuations will affect soil moisture availability, which is an important driver of soil microbial activity and carbon dioxide release. However, the question remains if the legacy of one type of fluctuation influences the microbial response to the other. We hypothesized that freezing-thawing and drying-rewetting cycles have similar effects on the soil microbial community and soil respiration. Three microcosm experiments were performed using grassland soil from the Netherlands, where we had knowledge of prior climate. In the first experiment, we exposed soil microbes to a freeze-thaw or a drying-rewetting cycle and control treatments. The second and third experiment consisted of two phases. In the first phase, soil microcosms were exposed to a similar treatment as in experiment one. In the second phase, soil microcosms were exposed to the a drying-rewetting or freezing-thawing cycle. We measured soil respiration and extracted RNA to investigate the potentially active fraction of the microbial community using amplicon-sequencing. We observed a larger CO₂ pulse upon rewetting than upon thawing. Importantly, the legacy of the drying-rewetting treatment affected the response of the microbial community and CO₂ emissions upon the second freezing-thawing event. In contrast, the legacy of the freezing-thawing event did not affect the response of the microbial community to a drying-rewetting event. Our results suggest that drying-rewetting has a bigger influence on soil microbial communities than freezing-thawing and leaves a legacy in the soil microbial community.

Taxon-specific assimilation of different N sources by forest soil microorganisms

David Myrold^{*1}, Peter Bottomley¹, Kristin Kasschau¹, Xavier Mayali², Daniel Nilson², Jennifer Pett-Ridge³

¹Oregon State University, United States, ²Lawrence Livermore National Laboratory, United States, ³Lawrence Livermore National Laboratory, United States

All microorganisms need and assimilate nitrogen (N) for growth and activity, but this part of the N cycle has largely been ignored by microbial ecologists compared to processes done by just a subset of microorganisms, such as nitrification and denitrification. Our objective was to determine if different microbial taxa display preferences for different forms of N, including ammonium (NH_4^+), nitrate (NO_3^-), and amino acids. We focused on bacteria and archaea that were present in forest soils that varied in relative N availability and used the Chip-SIP method (where RNA isotopic enrichment is measured on a phylogenetic microarray with an imaging mass spectrometer) to determine the N assimilation preferences of the most important bacterial and archaeal taxa. Short-term laboratory incubations of soil were done in the presence of ^{15}N -labeled NH_4^+ , NO_3^- , or glycine. Total RNA was extracted when assimilation peaked, hybridized to the custom probe array, and assayed for the degree of ^{15}N labeling using nanoSIMS analysis to determine taxon-specific assimilation of the added ^{15}N -labeled compounds. Most taxa were able to use all of the N sources, with the overall order of preference being: glycine> NH_4^+ > NO_3^- ; however, the most actively assimilating taxa varied between sites and among N sources. These data demonstrate that diversity exists among microbial taxa in the universal function of N assimilation. Such diversity in N assimilation may influence interactions among members of the soil microbial community.

Genome-resolved metagenomic analysis of microbial communities in soils along a natural climatic gradient in the Scandinavian Arctic

Igor S Pessi*, Sirja Viitamäki, Jenni Hultman
University of Helsinki, Finland

Increased soil thawing due to pronounced warming in high northern latitudes might lead to a positive warming feedback loop, as previously frozen organic matter is metabolized by microorganisms releasing carbon dioxide and methane. Nevertheless, microorganisms involved in the production of greenhouse gases are not well known and a more thorough knowledge on the functional potential of microbial communities in Arctic soils is needed. We applied a genome-resolved metagenomic approach to investigate the functional potential of microbial communities in 50 soil plots along a natural climate gradient in the Scandinavian Arctic. Read-based analyses revealed communities dominated by Proteo-, Actino- and Acidobacteria and evidenced a high proportion of genes involved in the breakdown of aminoacids, carbohydrates and lipids. A first assembly of reads from a subset of the samples yielded 10 metagenome-assembled genomes with >70% completion and <5% redundancy. Some were related to common soil taxa such as the nitrogen-fixing *Bradyrhizobium* (Alphaproteobacteria) and *Granulicella* (Acidobacteria), while others appear to represent distinct lineages distantly related to the genera *Eliaera* (Alphaproteobacteria), *Candidatus Koribacter* (Acidobacteria) and *Thermobifida* (Actinobacteria). Further phylogenetic and functional analyses of the retrieved genomes are being currently carried out, and assembly of the remaining samples will potentially result in the recovery of additional genomes. These analyses will allow us to investigate the metabolic potential of the soil metagenomes in more detail, for example, regarding the importance of carbon,

nitrogen and sulfur cycles in the studied soil ecosystem along fine-scale climatic variation.

Comammox *Nitrospira* in volcanic grassland soils – changes in abundance in response to a natural soil warming gradient

Petra Pjevac*, Andreas Richter, Holger Daims, Michael Wagner
University of Vienna, Austria

Nitrification, a key process of the biogeochemical nitrogen cycle, strongly affects nitrogen availability in native and agricultural soils, and contributes to emissions of the greenhouse gas nitrous oxide (N₂O). The distribution, diversity and abundance of canonical nitrifiers - ammonia oxidizing bacteria and archaea (AOB and AOA), and nitrite oxidizing bacteria (NOB) - has been extensively studied in terrestrial environments. However, very little is known about the recently discovered complete ammonia oxidizers (comammox) and their response to environmental changes.

In this study we assessed the diversity and abundance of comammox *Nitrospira* in volcanic grassland soils from Iceland. The investigated sites represent transects over natural soil warming gradients (up to 6°C above ambient temperature) undergoing geothermal heating for at least 10 years. Previous studies showed AOA to be highly abundant in similar soils, whereas AOB were undetected by molecular assays. In freshly recovered soil samples, we confirmed a high abundance of AOA and did not detect AOB. Ammonia monooxygenase subunit A (*amoA*) genes of comammox *Nitrospira* from clade B were highly abundant, suggesting that comammox *Nitrospira* are important but previously overlooked nitrifiers in volcanic grassland soil. Furthermore, a significant increase in comammox *Nitrospira*-related *amoA* gene copy numbers was observed with increasing temperature, whereas the copy numbers of AOA-related *amoA* genes did not vary significantly. Hence, warming had a pronounced effect on the nitrifying communities with possible consequences for nitrous oxide emissions. Our results are first steps towards elucidating the role of comammox *Nitrospira* in the context of terrestrial nitrogen cycling and global change.

It's activity not growth: Quantifying milli-niches using positron emission tomography of psychrotrophic bioremediation in permafrost soils

Steven Siciliano*¹, Yu-Fen Chang², Alix Conway¹, Whitney Shannon¹, Steven Mamet¹, Bobbi Helgason³, Derek Peak¹, Alireza Talebitaheer², Zisis Papandreou², Tom Palaia⁴, Aram Teymurazyan²

¹University of Saskatchewan, Canada, ²University of Regina, Canada, ³AAFC, Canada, ⁴CH2M Hill, United States

Soil ecosystems are managed on a landscape scale, but soil microbial communities operate on a millimetre scale. The combined activity and distribution of these

innumerable microscale communities gives rise to ecosystem services that protect groundwater, regulate climate, and/or produce food. Recent advances in detector technology, now make it possible to image microbial distribution and activity, at the millimetre scale throughout intact field soil cores. Using positron emission tomography (PET), we evaluated if a novel biostimulatory solution would allow isolated Arctic communities to bioremediate hydrocarbon polluted soils in situ. We characterized microbial distribution, activity, gene expression, metabolism, and composition in 15 different boreholes collected from the permafrost microbial community. In soil volumes of ca. 48 cm³, microorganisms filled approximately 60% of soil pore space (ca. 17 cm³), with the remaining pore space (ca. 10 cm³) filled with gas or water. Biostimulated microbial communities metabolized approximately 26% of the Fludeoxyglucose (¹⁸F) tracer, whereas control microbial communities only metabolized 11%. Microbial community distribution in situ was not necessarily linked to the active soil pores, with microbial communities completely filling pore areas in which the non-biologically reactive tracer, ¹⁸F, could not. The in situ distribution of soil microbial communities was linked ($r=0.45$, $p<0.05$, $n=80$) to traditional measures of gene expression, pollutant degradation and basal respiration. Despite higher metabolism, pollutant degradation, 16S rRNA abundance, and gene expression, biostimulated community biomass was not increased compared to control communities. In situ remediation under psychrotrophic conditions works by stimulating microbial activity but not growth.

Disturbance intensity alters soil microbial community reassembly dynamics

Simone Weidner^{*1}, Joost Keuskamp¹, Hylke Kortenbosch², Wu Xiong¹, Rong Li³, George Kowalchuk¹, Alexandre Jousset¹

¹*Utrecht University, Netherlands*, ²*University of Groningen, Netherlands*, ³*Nanjing Agricultural University, China*

Ongoing climate change is leading to an increase in frequency of extreme weather events. These disturbances can affect soil microbial communities, thereby impacting crucial functions related to plant health and ecosystem functioning.

We studied effects of disturbance intensity on post-disturbance microbial regrowth dynamics and community functioning. We mimicked extinction of varying intensity by re-inoculating sterilized soil with its own serially diluted microbial extract. We explored post-disturbance effects at three levels of integration: soil microbial community composition, assembly on plant roots and functioning of bacterial isolates.

Nine weeks after inoculation microbial biomass had recovered. Dilution negatively affected potential growth rate and carbon resource use, but increased the carrying capacity. Increasing disturbance thus appears to select for yield, as opposed to growth, strategists. Dilution also decreased bacterial diversity, whilst increasing soil bacterial community variability.

Dilution effects on the bulk soil bacterial community were propagated to subsequent rhizosphere communities. Dilution decreased bacterial diversity whilst increasing

negative interactions (correlation network analysis). This result is in line with a decline in the production of public goods by bacterial isolates with increasing dilution (siderophore production, antibiotic production).

Together, our results show that extinction events reshape soil microbial communities by creating shifts in the dominant life history strategies, promoting selfish, antagonistic and yield- oriented microorganisms. We therefore expect that extreme weather events may have long-lasting effects on soil functioning even after apparent recovery.

What drives the assembly of plant-associated protist microbiomes?

Michael Bonkowski^{*1}, Kenneth Dumack¹, Anna Maria Fiore Donno¹, Susanne Schreiter², Rita Grosch³, Kornelia Smalla²

¹*University of Cologne, Germany*, ²*Julius Kühn-Institut, Germany*, ³*Leibniz Institute of Vegetable and Ornamental Crops Großbeeren/Erfurt e.V., Germany*

The rhizosphere microbiome is important for plant growth and health, but dissecting the factors that shape this microbiome in the rhizosphere is still challenging. We investigated the influence of soil type and plant species on the community composition of bacteria and bacterivorous protists (Cercozoa: Rhizaria) in the rhizosphere of lettuce and potato plants. This study differs from previous research in two important aspects: i) we included with bacterial consumers a further trophic level for microbiome assembly, and ii) both plant species were grown under field conditions in a unique experimental plot system with three soil types at the same field site. The importance of protist predators for bacterial community composition in the rhizosphere has been well established in laboratory experiments. We hypothesized that this top-down control together with the bottom-up control of bacterial communities via rhizodeposition provides a crucial feed-back for microbiome assembly. Soil type and plant species acted as strong environmental filters for bacterial and protist community composition. The cercozoan distribution patterns showed compelling similarities to the patterns observed for the bacteria. Accordingly, bacterial rhizosphere communities exerted an important feedback on protist community assembly, indicating the existence of a new self-regulating mechanism for microbiota on plant roots.

Fine scale niche differentiation among amoebal communities of dark-spored myxomycetes determined by landscape structures as well as biotic factors

Mathilde Borg Dahl^{*1}, Asker Brejnrod², Jakob Russel², Søren Sørensen², Martin Schnittler¹

¹*Ernst Moritz Arndt University Greifswald, Germany*, ²*Department of Biology, University of Copenhagen, Denmark*

Myxomycetes (Myxogastria, Amoebozoa) also known as 'slime molds' have historically been described as fungi, due to their macroscopically visible fructifications which, though considerably smaller, resemble those of fungi. These fruit bodies provide enough morphological traits to support a morphological species concept with currently ca. 1000 species described. Therefore diversity studies of myxomycetes have been conducted for over 200 years and a substantial body of data on ecology and distribution of these fructifications exist. In the study presented we ask: 'do myxomycetes show broader realized niches as soil amoebae than as fructifications?' and 'is the myxamoebae distributions correlated to potential prey organisms (fungi and/or bacteria)?'. To answer these questions parallel metabarcoding of bacteria, fungi and dark-spored myxomycete was used in a combined approach to analyse the communities from an elevational transect in the northern limestone German Alps (48 soil samples). Potential interactions between the three target organisms were analysed by integrating community composition and

phylogenetic diversity with environmental parameters. We identified niche differentiation for all three communities (bacteria, fungi and myxamoebae) which was strongly driven by the vegetation. Bacteria and fungi displayed similar community responses, driven by symbiont species and plant substrate quality. Myxamoebae showed a more patchy distribution, though still clearly stratified among genera, which seemed to be a response to both structural properties of the habitat and specific bacterial taxa. In addition we find an altitudinal species turn-over for all three communities, most likely explained by adaptation to harsh environmental conditions.

Protozoan egested food vacuoles are an unrecognized vector for the transmission of cholera

Gustavo Espinoza-Vergara¹, Parisa Noorian², Cecilia Silva-Valenzuela³, Benjamin Raymond⁴, Christopher Allen⁵, Shuyang Sun⁴, Staffan Kjelleberg⁶, Maurizio Labbate⁵, Steven Djordjevic⁷, Andrew Camilli⁸, Diane McDougald^{*4}

¹University of New South Wales, Australia, ²School of Biotechnology and Biomolecular Science, University of New South Wales, Sydney, 2052 Australia, Australia, ³Department of Molecular Biology and Microbiology, Tufts University, School of Medicine, Boston, MA 02111, USA, United States, ⁴The itthree Institute, University of Technology Sydney, Sydney 2007, Australia, Australia, ⁵School of Life Sciences, University of Technology Sydney, Sydney 2007, Australia, Australia, ⁶Singapore Centre for Environmental Life Sciences Engineering, School of Biological Sciences, Nanyang Technological University, Singapore 637551, Singapore, ⁷The itthree Institute, University of Technology Sydney, Sydney 2007, Australia, Australia, ⁸Department of Molecular Biology and Microbiology, Tufts University, School of Medicine, Boston, MA 02111, United States

Bacterivorous protists (protozoa) frequently release undigested bacteria in egested food vacuoles (EFV), which have, until now, been an unrecognized vector for the transmission of cholera. Here we used confocal microscopy, exposure to stresses (pH and antibiotics) and the infant mouse model of colonization to investigate stress resistance and infection potential of cells contained in the EFVs.

Our results show that the protozoan ciliate *Tetrahymena pyriformis* releases large numbers of EFVs when feeding on *Vibrio cholerae*. EFVs were demonstrated to be extremely stable in artificial seawater with no significant loss of viability after long term storage at room temperature. Cells within the EFVs were not affected by incubation at low pH (3.4) or in the presence of antibiotics. When incubated at 37°C in the presence of nutrients, the cells escaped very rapidly. Escaped cells were shown to have a fitness advantage over planktonic cells both *in vitro* and *in vivo*. These findings suggest that EFVs could facilitate the survival and ensure the persistence of *V. cholerae* in the environment under a variety of stressful conditions and that the EFVs would also protect *V. cholerae* as it transits through the stomach. In addition, the escaped cells have enhanced colonization potential. The work also shows that cells within EFVs are primed to cause infection and may be a significant contributor to the dissemination of epidemic *V. cholerae* strains.

Secret but important life of picocyanobacteria in hypertrophic waters

Jitka Jezberová^{*1}, Anna Matoušů¹, Karel Šimek¹, Jiří Nedoma¹, Jaroslav Vrba²

¹*Biological Centre CAS - Institute of Hydrobiology, Czech Republic*, ²*University of South Bohemia – Faculty of Biological Sciences, Czech Republic*

Although picocyanobacteria of the *Synechococcus-Cyanobium* group have been studied intensively in oligotrophic waters, they are heavily underestimated and neglected in eutrophic and hypertrophic waters. On a large set of hypertrophic fishponds, that are used for intensive aquaculture, we have demonstrated that picocyanobacteria are very abundant and important here. In some cases they reached up to 10^7 cells per ml and played a key role in the trophic chain. Each living form had different predators and different function in the trophic chain. The single-cell form in the spring was grazed by protozoa (especially by several different kind of ciliates) and the colonial in summer was heavily grazed by zooplankton (especially by nauplius and *Daphnia*). Microbial and picocyanobacterial genetic diversity was investigated by next-generation-sequencing. This research is very important for understanding of the complex view on functioning of hypertrophic fishpond systems and the nutrient flow from sediment to upper trophic layers.

Mitigating Soil N₂O Emissions by Fungivorous Mites

Haoyang Shen^{*1}, Yutaka Shiratori², Sayuri Ohta², Keishi Senoo³

¹*Graduate School of Agricultural and Life Sciences, The University of Tokyo, Japan*, ²*Niigata Agricultural Research Institute, Japan*, ³*Graduate School of Agricultural and Life Sciences, Collaborative Research Institute for Innovative Microbiology, The University of Tokyo, Japan*

Nitrous oxide (N₂O) is an important long-lived greenhouse gas. N₂O emissions from soils are mainly resulted from microbial processes including denitrification and nitrification of soil bacteria, archaea and fungi. However, the involved ecological processes are largely unknown. Here, we show that the fungivorous mite, a dominant group of soil mesofauna, plays an important role in regulating soil N₂O emissions by feeding on N₂O-producing fungi. In a sweetcorn field applied with organic fertilizer, we observed a considerable decrease of N₂O emissions and an increase of fungivorous mite abundances after coconut husk (in chips), a natural soil conditioner, was applied to the soil. However, no N₂O mitigation was observed when husk was applied together with miticide. Therefore, we hypothesized that by increasing fungivorous mite abundances, husk application to soils would suppress the growth of N₂O-producing fungi and thus mitigate N₂O emissions. Two microcosm experiments were performed to verify this hypothesis. Firstly, when 40 mites were applied to 200 g of soil, decreases of fungal 18S rRNA gene abundances by 32% and N₂O emissions by 36% were observed, indicating a N₂O mitigation caused by fungi consumptions by mites; Secondly, when 1 g of husk was applied accompanying with mites, a 30% lower N₂O emission and a 77% higher mite

abundance were obtained compared to the soil applied with only mites, indicating an enhancement of mite reproduction by the husk application. Our findings reveal an ecological relationship between fungivorous mites and N₂O emissions, which may provide a new strategy for mitigating soil N₂O emissions.

Nematode grazing promotes bacterial community dynamics in a red soil at the aggregate level

Bo Sun^{*1}, Yuji Jiang¹, Manqiang Liu², Yan Chen¹, Huixin Li², Xue-Xian Zhang³
¹*Institute of Soil Science, Chinese Academy of Sciences, China*, ²*Nanjing Agricultural University, China*, ³*Institute of Natural and Mathematical Sciences, Massey University, New Zealand*

Nematode predation plays important roles in determining bacterial community composition and dynamics, but the extent of the effects remains largely rudimentary, particularly in natural environment settings. Here, we investigated the complex microbial-microfaunal interactions in the rhizosphere of maize grown in red soils, which were derived from four long-term fertilization regimes. Root-free rhizosphere soil samples were separated into three aggregate fractions whereby the abundance and community composition were examined for nematode and total bacterial communities. A functional group of alkaline phosphomonoesterase producing bacteria was included to test the hypothesis that nematode grazing may significantly affect specific bacteria mediated ecological functions, i.e. organic phosphate cycling in soil. Results of correlation analysis, structural equation modelling and interaction networks combined with laboratory microcosm experiments consistently indicated that bacterivorous nematodes enhanced bacterial diversity, and the abundance of bacterivores was positively correlated with bacterial biomass, including ALP-producing bacterial abundance. Significantly, such effects were more pronounced in large macroaggregates than in microaggregates. There was a positive correlation between the most dominant bacterivores *Protorhabditis* and the ALP-producing keystone “species” *Mesorhizobium*. Similarly, our previous works revealed that this stratification effect of nematodes predation displayed the positive effects on total bacteria in bulk soils, as well as ammonia-oxidizing bacteria (especially for the dominant genus *Nitrosospora*). Taken together, these findings implicate important roles of nematodes in stimulating bacterial community dynamics in a spatially dependent manner.

Grazing pressure induced shift in planktonic community structure leads to the unusual dominance of acIII actinobacterial lineage in alkaline soda pans

Attila Szabo^{*1}, Kristof Korponai², Zsuzsanna Marton², Zsolia Horvath³, Boglarka Somogyi⁴, Lajos Voros⁴, Emil Boros⁴, Karoly Marialigeti², Tamas Felfoldi²
¹*Department of Microbiology, ELTE Eotvos Lorand University, Hungary*, ²*ELTE Eotvos Lorand University, Hungary*, ³*Wasser Cluster Lunz, Austria*, ⁴*MTA Centre for Ecological Research, Balaton Limnological Institute, Hungary*

Astatic soda pans of the Carpathian Basin are unique environments regarding their physical and chemical characteristics (high turbidity, pH and salinity coupled with ionic composition distinct from other saline lakes). The pans are highly productive environments with picophytoplankton dominance. Fish are absent from these lakes, but microcrustaceans, especially the natronophilic *Arctodiaptomus spinosus* (Copepoda: Calanoida) and *Moina brachiata* (Cladocera) can be abundant by late spring. In our study, two alkaline hyposaline soda pans, representing distinct ecological types (the turbid-type Zab-szék pan and the coloured-type Sós-ér pan), were sampled monthly between April 2013 and July 2014 to reveal seasonal changes in the structure of bacterioplankton and related these to changes in biotic and abiotic parameters. By late spring/early summer in both years, a sudden change in the community structure was observed in the two lakes. The previous algae-associated Flavobacteriia-, Cytophaga- and Rhodobacterales-dominated communities collapsed, and Actinobacteria, characterized by the acIII lineage, became dominant, with sometimes up to 88% relative abundance within the communities. Before the actinobacterial peaks, extremely high abundance (>10 000 ind per litre) of microcrustaceans were observed. Previous studies showed that planktonic actinobacteria are more resistant to grazing than other freshwater bacteria due to their small cell size and the presence of S-layer in their cell wall. OTU-based statistical approaches showed that besides algal blooms and water-level fluctuations, zooplankton densities had the strongest effect on the composition of these bacterial communities, which implies a strong community shaping role of microcrustacean grazers.

A bet-hedging strategy during the shift to anaerobic respiration curtails N₂O emissions

Linda Bergaust^{*1}, Pawel Lycus¹, Manuel Soriano-Laguna², Morten Kjos¹, David Richardson², Andrew Gates², Daniel Milligan¹, Åsa Frostegård¹, Lars Reier Bakken¹
¹Norwegian University of Life Sciences, Norway, ²University of East Anglia, United Kingdom

When conditions become anoxic, denitrifying prokaryotes switch to anaerobic respiration using nitrogen oxides as terminal electron acceptors. The past decade has seen an expansion in our knowledge about the vast diversity in the regulation of this process. This has profound implications for our understanding of the role of denitrifiers in the release of NO and N₂O to the atmosphere. Here, we describe bet-hedging during the shift between aerobic respiration and denitrification in the well-known model organism *Paracoccus denitrificans*. In cultures experiencing hypoxia, all cells synthesize N₂O reductase (N₂OR), whereas only a minority synthesizes nitrite reductase (NIR). Such cultures are strong net sinks of N₂O. To trace NIR in *P. denitrificans*, we constructed a strain where the *nirS* gene was replaced by a chimeric *mCherry-nirS* fusion gene under the control of the same promoter, facilitating tracking of NirS positive cells by red fluorescence. To detect N₂OR in single cells, we developed an immunofluorescence staining method, enabling its visualization in individual cells. We also developed a FITC stain dilution assay to track cell growth. This approach allowed us to demonstrate phenotypic diversification in our model organism. The initial hypothesis was based on modelling respiratory lags after oxygen depletion. Such lags were observed in a number of organisms during the transition to anoxia, suggesting that bet-hedging is widespread. Moreover, the phenotype was pronounced at temperatures relevant to soil (< 20°C) and less so at higher temperatures, underscoring the potential environmental significance of this novel regulatory phenomenon.

MetaSUB International Consortium: creating the global microbiome portrait of the world's urban systems, one city at a time

Aspa D. Chatziefthimiou^{*1}, Isha Lamba¹, Ajay Menon¹, Shahryar Rana¹, Salama Chaker¹, The MetaSUB International Consortium², Christopher Mason²
¹Weill Cornell Medicine - Qatar, Qatar, ²Weill Cornell Medicine - New York, United States

MetaSUB was founded in 2015 as a global, transparent, and integrated metagenomics initiative, bringing together scientists, bioinformaticians, medical practitioners, industrial partners, government officials and artists. MetaSUB collaborative efforts aim to inform work in city planning, urban design, transit systems, public health, ecological studies, and to improve metagenomics technologies and visualization methods. MetaSUB is creating geospatial molecular maps of mass-transit systems in the built environment in over 70 cities around the world. Recent technologies and algorithms developed by our consortium partners and others demonstrate that an integrative, cross-kingdom view of patients (precision metagenomics) holds unprecedented biomedical potential to discern risk,

improve diagnostic accuracy, and to map both genetic and epigenetic states. Leveraging these data, the global profile of the world's urban systems is being created to track the intra-city and inter-city shifts in antimicrobial resistance (AMR) markers. Furthermore, geospatial molecular variations correlated with environmental metadata, shed light on the underlying causes of biogeographical patterns of microbial communities and how these respond and adapt to changing climate. Here we present results from over 13,000 environmental DNA samples collected during two Annual Global City Sampling Days in 2016 and 2017. Based on our data analysis so far, we observed that each city has unique community composition patterns as well as signatures of AMR density and movement, and that key drivers of these differences are climate-dependent and match the rate of antibiotic use in those countries. This is the first study to provide the genetic map of DNA and AMR exchange in urban environments.

Investigating organic substrate utilization by marine Thaumarchaeota using metatranscriptomics and nanoscale secondary ion mass spectrometry

Anne Dekas^{*1}, Alma Parada¹, Xavier Mayali², Jed Fuhrman³, Peter Weber², Jennifer Pett-Ridge²

¹Stanford University, United States, ²Lawrence Livermore National Laboratory, United States, ³University of Southern California, United States

Thaumarchaeota are nearly ubiquitous in marine and terrestrial habitats, and likely play an important role in global biogeochemical cycles. They are thought to live primarily chemoautotrophically, coupling ammonia oxidation to bicarbonate fixation. However, some evidence suggests that they may have heterotrophic or mixotrophic capabilities. We investigated the ability of marine Thaumarchaeota to assimilate organic substrates *in situ* using metatranscriptomics and isotope tracer experiments analysed by fluorescence *in situ* hybridization coupled to nanoscale secondary ion mass spectrometry (FISH-nanoSIMS). We incubated water collected at 150 meters depth at the San Pedro Ocean Times Series (SPOT) station with ¹³C- and ¹⁵N-labelled organic substrates. Illumina amplicon sequencing of the 16S rRNA gene indicated an abundant and active Thaumarchaeal population: Thaumarchaeal OTUs comprised 15% of DNA reads and 60% of cDNA reads. Metagenomic analysis showed that Thaumarchaeal metagenome-assembled genomes (MAGs) contained genes associated with uptake or processing of organic substrates (e.g., amino acids, carbohydrates, lipids), and metatranscriptomic analysis demonstrated that many of these genes were expressed *in situ*. In contrast, only minor organic carbon assimilation was observed by single-cell resolution FISH-nanoSIMS: some carbon was assimilated from urea and amino acids in a subset of the Thaumarchaeal cells, and no uptake was observed from glucose, pyruvate, oxaloacetate, or proteins. Intraphylum heterogeneity and/or the use of nitrogenous organic matter as a source of ammonia for oxidation may explain the seemingly conflicting results observed here and in previous studies. Our results highlight the value of combining -omic analyses with single-cell imaging of microbial activity.

Growing in spatial collectives helps *Caulobacter crescentus* cells to utilise complex plant derived polysaccharides

Glen Dsouza*, Martin Ackermann
ETH Zurich and Eawag, Switzerland

In nature, bacterial cells reside within spatial assemblages and metabolize complex plant and animal derived nutrients like polysaccharides to fuel essential cellular processes. As a consequence, growth and metabolism of cells brings about a transformation in the nutrient environment, which can strongly influence the behavior of cells residing in the same microenvironment. What bacterial growth behaviors manifest in the presence of such complex resources like polysaccharides? And how does behavior of individuals influence other cells in bacterial assemblages? Here, we use microfluidics and time-lapse microscopy to study spatial and behavioural growth dynamics of the freshwater bacterium *Caulobacter crescentus* on xylan, a ubiquitous plant derived polysaccharide composed of xylose units. In presence of xylan, newly divided cells stay in close proximities of each other, thus effectively increasing size of a cell's neighborhood. In contrast, cells grown with xylose lead solitary lives. Furthermore, a transition of the environments from xylan to xylose results in initially spatially associated cells to disperse and engage in solitary lifestyles. We find that such collective behavior in the presence of xylan is beneficial for cells to avoid a diffusional loss and increase the availability of both, the exoenzyme and end product: i.e. xylanase and xylose, respectively. A transition to a solitary state only ensues when such benefits are no longer needed. Our results elucidate an important and potentially ubiquitous microbial growth strategy in motile bacteria and further the understanding of how bacterial cells adjust their behaviors to adapt to dynamic and often self-imposed changes in the environment.

***In situ* activity and metabolism of uncultured thermophiles experimentally determined at single cell resolution through Next Generation Physiology**

Roland Hatzenpichler*, Nick Reichart, Viola Krukenberg, Rachel Spietz
Montana State University, United States

Enabled by the rise of genomics, microbial ecology studies often produce a large collection of hypotheses on the physiology of microbes that only rarely are empirically tested. An obstacle to pursuing such experiments on a regular basis is that existing techniques often necessitate to target only a specific species or functional guild, while other members of the microbial community are ignored.

To overcome this problem, we developed a new, parallelizable, and replicable approach we call Next Generation Physiology. It allows functional activities and ecological niches of uncultured cells to be studied on systems-wide scale at yet unrivaled throughput and in an easily adaptable manner. Conceptually, we combine the incubation of natural samples under a variety of conditions with the labeling of translationally active cells by bioorthogonal non-canonical amino acid tagging. We

then separate active from inactive cells by fluorescence-activated cell-sorting and characterize them by massive parallel gene-sequencing.

We used our approach to study the metabolism of microbes in an alkaline (pH8.5) geothermal (71°C) spring in Yellowstone National Park. We tested the activity of poorly characterized (candidate) phyla, including *Aigarchaeota*, *Armatimonadetes*, GAL08, KB1, and *Parcubacteria*, under 34 different conditions. We revealed OTU-specific adaptations to 10 carbon-sources and contrasting activities under different oxygen-concentrations, temperatures, and vitamin availabilities. In contrast to expectations, several abundant OTUs exhibited highest activities under conditions not prevailing at time of sampling. In order to link specific pathways with *in situ* function, we now are starting to analyze the genomes of sorted, active cells from a subset of incubations.

Cellular biomass to volume relationship for aquatic microorganisms

Alexander Khachikyan^{*1}, Sten Lettmann¹, Jana Milucka¹, Thomas Burg², Marcel M. M. Kuypers¹

¹Max Planck Institute for Marine Microbiology, Germany, ²Max Planck Institute for Biophysical Chemistry, Germany

The correct estimation of biomass for aquatic microorganisms is essential for determining their relevance in biogeochemical cycling and ecology. Since direct biomass measurements are difficult, cell biomass is often estimated using empirically derived biomass:volume relationships. These relationships have been determined for a wide range of organisms, but large discrepancies occur in biomass estimation of small cells (<1 μm^3), mainly due to methodological issues in measuring nutrient content in microbial cell size. However, many cells of this size play a key role in the biogeochemical cycling of carbon and nitrogen, such as archaea *Nitrosopumilus maritimus* in ammonium oxidation and anammox bacteria *Kuenenia stuttgartiensis* in nitrogen loss.

We measured cell volumes and elemental compositions of twelve bacterial and archaeal species with volumes of individual cells ranging from ca. 0.004 μm^3 to 1.4 μm^3 . Dry weight of single microbial cells was determined using suspended microchannel resonator. Cell volumes were determined using scanning electron microscopy and elemental composition was measured using energy-dispersive X-ray spectroscopy. We found significant allometric relationships between carbon and nitrogen mass and cell volume. More specifically, our results show that smaller cells have more carbon and nitrogen per cell volume than larger cells. This implies that currently used biomass:volume relationships for cellular carbon and nitrogen content calculation underestimate the content of these elements in small cells, and thus lead to an underestimation of their contribution to biogeochemical carbon and nitrogen cycling.

Using DUV Raman and fluorescence spectroscopy to localize biomass and identify microbial activity

Haley Sapers^{*1}, Rohit Bhartia², Jan Amend³, Brittany Kruger⁴, Sean Mullin⁵,
Magdalena Osburn⁶, Greg Wanger¹, Victoria Orphan⁵

¹*California Institute of Technology/ NASA Jet Propulsion Laboratory/ University of Southern California, United States*, ²*NASA Jet Propulsion Laboratory, United States*, ³*University of Southern California, United States*, ⁴*Desert Research Institute, United States*, ⁵*California Institute of Technology, United States*, ⁶*Northwestern University, United States*

Microbial communities living in close association with lithic substrates play critical roles in biogeochemical cycles. Understanding the interactions between microorganisms and their substrates requires knowledge of the community structure and assessment of the spatial distribution of metabolic activity. Stable isotope probing (SIP) provides a means to trace microbial metabolic activity in environmental systems. Here we show that Deep UV (248.6 nm) Raman spectroscopy can differentiate bacterial cells grown in isotopically labeled media. A combined DUV Raman-SIP approach has the potential to identify microbial activity while obtaining the spatial community structure relative to the environmental substrate through correlated DUV fluorescence spectroscopy mapping. DUV Raman leverages resonance and pre-resonant enhancement of vibrational modes in aromatic organic molecules to provide fundamentally different information compared to under visible (i.e. green Raman) excitation. Furthermore, DUV excitation results in Raman emission in a fluorescence-free window, allowing for detection of organic material on mineral surfaces that would otherwise be obscured by intense background fluorescence. *In situ* field incubations of mineral substrates in a deep continental setting (4850' level, Sanford Underground Research Facility, South Dakota, USA) illustrate the ability of scanning DUV fluorescence spectroscopy to localize and visualize biomass on environmental substrates. Following incubation, DUV fluorescence was used to identify areas of colonization. The ability of DUV Raman to effectively identify regions containing cells that have incorporated isotopic labels combined with targeted fluorescence mapping will facilitate *in situ* detection of metabolically-targeted active community members on natural mineral substrates, providing a crucial link between microbial activity and environmental context.

A Swiftian Voyage from Brobdingnag to Lilliput: Freshwater Planctomycetes drifting towards the poles of the genome size spectrum

Stefan Andrei*¹, Michaela Salcher², Maliheh Mehrshad¹, Rohit Ghai¹

¹*Biology Centre of the ASCR, Institute of Hydrobiology, Czech Republic,* ²*Department of Limnology, Institute of Plant Biology, Switzerland*

Although prokaryotes dwell in virtually every spot that can sustain life, little is known about their evolutionary history and the genomic mechanisms through which they colonize new habitats. Here, we use genome-resolved metagenomics (60 genomes recovered from ten large metagenomic datasets) and ecosystem-scale taxonomic profiling (299 metagenomes) in order to elucidate the origins of freshwater Planctomycetes and link their genome evolution patterns with their lifestyle strategies. The evolutionary history inferences indicated that sediment/soil Planctomycetes transitioned to aquatic environments where, through processes mostly associated with reductive genome evolution, they gave rise to new freshwater-specific lineages. We show that these ancestral genomes underwent a biphasic process of evolution, in which an initial phase of innovation was succeeded by one marked by gradual loss of genetic material. Thus, the innovation stage represented the trigger for the habitat transition (which at this step may be bidirectional), as the acquisition of new gene sets led to increased metabolic versatility and paved the way for niche expansion. As these Planctomycetes entered the second (and much longer) evolutionary stage, the acclimatization to freshwater took the form of adaptive streamlining. We demonstrate that the most successful freshwater-specific lineage has simultaneously the most specialized lifestyle (increased regulatory genetic circuits; metabolism tuned for mineralization of proteinaceous sinking aggregates) and the smallest genome sizes. Such an evolutionary path (i.e. sediment/soil habitat transition coupled with reductive genome evolution and increased adaptability/success), might stand for a strategy widely spread in aquatic environments.

Heterotrophic substrate specificity in the aquatic environment: the role of microscale patchiness

Ferdi Hellweger

TU Berlin, Germany

Heterotrophic bacteria are important components of the aquatic carbon cycle with effects on climate and surface water quality, which makes understanding their ecology important. The existing belief, based on laboratory studies at low nutrient concentrations and common sense arguments, is that bacteria in the natural environment follow a mixed substrate use strategy. However, recent observations of natural bacterial communities from lakes and wastewater treatment plants show high genetic diversity in organic carbon uptake systems (microdiversity) and specificity in substrate species taken up. This apparent discrepancy can be cleared up by realizing that bacteria in the natural aquatic environment encounter nutrients as high-concentration patches. They may live in an ecologically nutrient-limiting

environment, but they are rarely in a biologically nutrient-limited state. Rather they switch between non-growing and nutrient-replete states. During nutrient-replete growth the metabolism is saturated and assimilating additional substrates does not increase the growth rate, but carrying the assimilation system constitutes a cost. Consequently, the specialist strategy is beneficial, which is consistent with observations from laboratory experiments. When the bacteria are not growing, the added cost also reduces the fitness of the generalist species. A simple mathematical model encompassing the relevant mechanisms is developed and parameterized realistically based on the literature. The model predicts that, under pulsed conditions, specialization is beneficial when the metabolic cost of an additional uptake system is more than ~0.5%, which is a reasonable estimate and illustrates that this is a plausible hypothesis.

Methane oxidizing bacteria revealed as major methane filter in submerged peat moss *Sphagnum* by using a novel mesocosm set-up

Martine Kox^{*1}, Martine Kox², Fons Smolders³, Daan Speth², Nardy Kip², Huub Op den Camp², Maartje Van Kessel², Mike Jetten²

¹Radboud University FNWI, dept. Microbiology, Netherlands, ²Radboud University, Netherlands, ³B-WARE, Netherlands

Wetlands are the largest natural source of methane emissions, emitting on average 164 gT yr⁻¹. In peatlands methane emission is in general lower than the potential emission, due to the activity of methane-oxidizing bacteria that act as a microbial biofilter. Until now, methane oxidation and production activity have been studied using peat soil slurries and net-production measurements in the field. In this study, we developed a novel mesocosm set-up that enabled exact control of methane input and monitoring of the dissolved methane throughout a peat column in a setting that closely resembled natural conditions. Two mesocosm set-ups were used in parallel: an experimental setup with a *Sphagnum* peat moss layer in peat water, and a control column containing only peat water. Over the 2-month course of the experiments, the methane oxidation rates increased by 50%. We were able to show that methane consumption only occurred in the peat moss layer and in the peat water, indicating that methane-oxidizing bacteria were only associated with the *Sphagnum* spp. FISH microscopy and qPCR of both 16S rRNA and pmoA genes, indicated that significant numbers of methane-oxidizing bacteria were present in and on the moss. Analysis of 16S rRNA amplicons revealed a diverse microbial community including type I and type II methanotrophs. Together these findings showed that our mesocosm setup can be used to study methane cycling in *Sphagnum* peat mosses under defined conditions. This is an important step forward towards better understanding of the interactions between methanotrophic communities and submerged *Sphagnum* mosses.

Autumn mixing in a seasonally stratified lake: good times for (some) methanotrophs

Magdalena J Mayr*, Matthias Zimmermann, Jason Dey, Andreas Brand, Helmut Bürgmann
EAWAG, Swiss Federal Institute of Aquatic Science and Technology, Switzerland

Stratified lakes with anoxic bottom waters may accumulate large amounts of methane, which might be released during autumnal lake mixing, potentially contributing up to 80% of the annual diffusive methane emission. The transition from a stratified to a mixed water column provides a natural whole lake “experiment” to study the response of methanotrophs to changing environmental conditions *in situ*. Until now, methanotrophic bacteria have mostly been investigated during the stratification period and not much is known about if and how methanotrophs react to seasonal lake mixing. From October 2016 to January 2017 we performed a detailed analysis of the development of the methanotrophic community and of physicochemical parameters in the water column of lake Rotsee, Switzerland. We sequenced bacterial 16S rRNA/rDNA and *pmoA* (subunit of the particulate methane monooxygenase) genes and transcripts and measured methane oxidation rates to follow the methanotrophic activity during mixing. We observed a gradual downward expansion of the mixed surface layer, which went along with a pronounced increase of methanotroph abundance and a community shift as some methanotrophs strongly increased in abundance, while others could not take advantage. Further, we show that otherwise rare methanotrophs can have their moment during the mixing period. Our study contributes to a better understanding of methanotroph ecology and methane oxidation dynamics in lakes. We demonstrate that the response of methanotrophs to autumnal mixing plays a role in regulating the greenhouse gas emissions from seasonally stratified lakes.

Contrasting microbial nitrogen-transforming networks in mountain lake benthic habitats

Carlos Palacin-Lizarbe*¹, Lluís Camarero², Sara Hallin³, Christopher M Jones³, Joan Caliz², Emilio O Casamayor², Jordi Catalan¹
¹CREAF, Spain, ²Center for Advanced Studies of Blanes, (CEAB-CSIC), Spain, ³Soil Microbiology Group, Department of Forest Mycology and Plant Pathology, Uppsala BioCenter, Swedish University of Agricultural Sciences, Sweden

Microbial nitrogen transforming networks are complex due to their modularity architecture and the metabolic versatility of the involved organisms. These networks are poorly known in freshwater oligotrophic ecosystems. We have studied them assessing by qPCR the abundances of nitrogen-functional genes (*nirS*, *nirK*, *nosZ* (clade I and II), archaeal and bacterial *amoA*, *nrfA*, and *hzo*) involved in denitrification, nitrification, DNRA and anammox pathways; and analyzing the community composition by sequencing the 16S rRNA gene in the main benthic habitats (lithic biofilms, sediments with elodeid and isoetid macrophytes, and littoral and deep non-vegetated sediments) of eleven mountain

lakes. The N-transforming networks are complex, with all the processes occurring alongside each other. Nonetheless, there is a marked diversity in the dominant pathways depending on the habitat with an associated and characteristic bacterial community. The fate of nitrite is the main diverging point, DNRA dominates in the deep part of the lakes, recycling the reactive-nitrogen; while denitrifying *nirS* nitrate reduction dominates in the surface sediments of the shallow, warmer and more productive lakes, losing reactive-nitrogen in these less oligotrophic systems. N₂ emissions may predominate in lithic biofilms, and N₂O in *nirS* dominated areas. There are two types of nitrifying-denitrifying coupled microbial networks. The dominant AOA/*Nitrospira* coupled to *nirS*-NO₂⁻ reduction and partially with *nosZII*-N₂O reduction, with hotspots in the rocky littoral sediments of the high-altitude alpine lakes and the buried sediments near the isoetid rhizosphere. The second type is more restricted to the lithic biofilms where AOB is coupled to *nirK*-NO₂⁻ and *nosZI*-N₂O reduction.

Microbial ecology and biogeochemistry of shallow groundwater aquifers

S. Emil Ruff, Pauline Humez, Isabella Hrabe de Angelis*, Sara Cho, Liam Connors, Michael Nightingale, Bernhard Mayer, Marc Strous
University of Calgary, Canada

Groundwater is a limited and vulnerable resource that is used for drinking water, agriculture and industrial applications. Baseline studies to understand the ecology of aquifers are crucial, to assess potential anthropogenic impacts for instance from industrial agriculture or the exploitation of hydrocarbon resources. Here we investigated the geochemistry and microbial ecology of groundwater samples from 120 monitoring wells located in southern and central Alberta, Canada, using total cell counts, gas and liquid chromatography, isotope ratio mass spectrometry, and meta-omic analyses. Elevated nitrate concentrations were observed in agriculturally intensive areas of Alberta, while the majority of the groundwater samples displayed sulfate-reducing or methanic redox conditions, containing dissolved methane of biogenic origin. The variabilities in groundwater chemistry were mirrored by differences in total cell counts and a high turnover between the microbial communities. The occurrence of certain electron donors or acceptors correlated with organisms involved in the cycling of these compounds, such as methanogens, methane oxidizers and sulfate reducers. We discovered a very high diversity of uncultured phylogenetic lineages. Many of these lineages are novel microbial phyla, however we also found unknown diversity in well-known clades such as aerobic methylotrophic *Methylococcales*. Overall, our study greatly expands our knowledge of subsurface microbial diversity and of the interactions between microbial food webs and groundwater biogeochemistry. Our results indicate biogeographical provinces in the terrestrial subsurface that share a similar chemistry and microbial ecology. This baseline dataset will be vital to inform future decisions on land use and to monitor long-term changes in groundwater ecology.

Complex phototrophic ecology in a seasonally anoxic Boreal Shield lake

Jackson M. Tsuji^{*1}, Sherry L. Schiff¹, Katharine J. Thompson², Sean A. Crowe², Kai Liu¹, Norman Tran¹, Jason J. Venkiteswaran³, Richard J. Elgood¹, Lewis A. Molot⁴, Josh D. Neufeld¹

¹University of Waterloo, Canada, ²The University of British Columbia, Canada, ³Wilfrid Laurier University, Canada, ⁴York University, Canada

Seasonally or permanently anoxic lakes frequently develop abundant populations of anaerobic photoautotrophs in the upper anoxic water column that can have substantial impacts on anoxic zone biogeochemistry. In most lakes, anaerobic photoautotrophs have been assumed to be sulfide-oxidizing bacteria. Here we present findings suggesting complex phototrophic ecology, involving multiple microbial taxa with differing modes of photosynthesis, in the anoxic water column of Lake 227, which is an artificially nutrient-amended, dimictic, and ferruginous Boreal Shield lake at the IISD-Experimental Lakes Area (IISD-ELA). Metagenomic data from hypolimnion samples collected over two summers show that the lake supports resilient populations of both sulfide- and iron-oxidizing *Chlorobi*. High-quality metagenome-assembled genomes of *Chlorobi* contained either the *dsrA* gene marker for sulfide oxidation or a *cyc2_{PV1}* gene homologue recently postulated as a gene marker for photoferrotrophy. In the narrow anoxic chemocline, measurements of iron oxidation rates suggest that oxygenic photosynthesis coupled to abiotic Fe(II) oxidation, rather than photoferrotrophy, is the dominant photosynthetic process, showing that pelagic oxygenic photoautotrophs can be active even in anoxic waters with nearly 100 μM dissolved iron. Sampled anoxic water columns of pristine IISD-ELA lakes show similar consortia of sulfide- and iron-oxidizing *Chlorobi* and oxygenic phototrophs, suggesting that this complex phototrophic ecology may be common throughout the Boreal Shield. Such photosynthetic ecological networks imply that multiple modes of photosynthesis may have operated contemporaneously in the ferruginous Archean and Proterozoic oceans, where the relative contribution of each form of phototrophy could have influenced global biogeochemical cycles and the dynamics of early Earth oxygenation.

Insights into antibiotic-resistance gene dissemination in dust microbiomes

Sarah Ben Maamar*, Adam Glawe, Taylor Brown, Ji-ping Wang, Erica Hartmann
Northwestern University, United States

The advent of next-generation sequencing has made it increasingly feasible to survey microbes in situ, including inside buildings where many people spend extended periods of time. Microbes in buildings, and specifically in dust, are linked to various health outcomes, and the amount of antimicrobial chemicals in dust is associated to the abundance of antibiotic resistance genes (ARG). However, it is unknown how many of the microbes we detect using DNA sequencing are in fact viable or whether it matters for the dissemination of ARG. To our knowledge, this is the first study to examine the potential for ARG dissemination within dust microbial communities.

Here, we used metagenomics to characterize dust microbial communities and their potential for ARG dissemination. Sequences were assembled, annotated and screened for integrons, transposons, plasmids and associated ARGs. These ARGs are being further investigated in the same dust samples for their potential presence and transferability via cultivation and molecular biology approaches.

In 167 dust metagenomes from 63 different buildings, we found 873 ARGs within 1075 bacterial taxa. Of those, 111 ARGs were mobile, i.e., on a plasmid, transposon or integron. Among mobile ARGs, the *gidB* gene conferring streptomycin resistance was exclusively found on plasmids in different dust samples. Screening for streptomycin-resistant isolates revealed several streptomycin-resistant strains (including *Staphylococcus*) that contain plasmids potentially carrying *gidB*. Presence and transferability of a mobile *gidB* gene in those strains are currently being investigated. This study sheds light on the presence and transferability of mobile ARG in the dust microbiome.

Genomic comparison of sulfonamide resistance mediated by a two component flavin-dependent monooxygenase system in sulfonamide-degrading actinobacteria

Chang-Jun Cha^{*1}, Dae-Wi Kim¹, Cung Thawng¹, Kihyun Lee¹, Elizabeth Wellington²
¹*Chung-Ang University, South Korea*, ²*University of Warwick, United Kingdom*

Sulfonamide-degrading bacteria have been discovered in various environments, suggesting the presence of novel resistance mechanisms via drug inactivation. In this study, *Microbacterium* sp. CJ77 capable of utilizing various sulfonamides as a sole carbon source was isolated from a composting facility. Proteome analysis revealed that a novel flavin-dependent monooxygenase named SulX paired with a flavin reductase was a key enzyme for the initial cleavage of sulfonamides. Co-expression of this two component system in *Escherichia coli* conferred decreased

susceptibility to sulfamethoxazole, indicating that the genes encoding drug-inactivating enzymes are potential resistance determinants. Comparative genomic analysis revealed that the *sulX* gene cluster was highly conserved in a genomic island shared among sulfonamide-degrading actinobacteria, all of which also contained *sul1*-carrying class 1 integrons. These results suggest that the sulfonamide metabolism may have evolved in sulfonamide-resistant bacteria which had already acquired the class 1 integron under sulfonamide selection pressures. Furthermore, the presence of multiple insertion sequence elements and putative composite transposon structures containing the *sulX* gene cluster indicated potential mobilization. This is the first study to report that *sulX* responsible for both sulfonamide degradation and resistance is prevalent in sulfonamide-degrading actinobacteria and its genetic signatures indicate horizontal gene transfer of the novel resistance gene.

Patterns of permissiveness towards broad host range plasmids in microbial communities across the urban water cycle in Europe

Arnaud Dechesne^{*1}, Liguang Li², Zhiming He², Joseph Nesme³, Marcos Quintela Baluja⁴, Sabela Balboa⁵, Jesús L. Romalde⁵, David Graham⁴, Søren J. Sørensen³, Barth F. Smets²

¹Technical University of Denmark / Env. Engineering, Denmark, ²Technical University of Denmark / Env. Engineering, Denmark, ³University of Copenhagen, Denmark, ⁴University of Newcastle, United Kingdom, ⁵University of Santiago de Compostela, Spain

Antimicrobial resistance genes are often carried by plasmids, which greatly facilitates their spread into microbial communities. Therefore, microbial community permissiveness (the propensity of a community to take up plasmids) and the diversity of the permissive members of the community constitute key parameters to understand and predict the fate of resistance genes. This is especially true for communities across the urban water cycle. Indeed, patients under antibiotics treatment excrete resistant microbes, which are collected and transported via the sewage collection network to wastewater treatment plants (WWTP). When these resistant, host-associated bacteria mix with other bacteria better adapted to life in the environment, there is a risk of transfer of resistance plasmids.

Here, we measured permissiveness towards three *gfp*-tagged model broad host range plasmids for communities at multiples points of the urban water cycle (hospital and residential sewers, influent of the WWTP, main WWTP reactor) in three European cities. Permissiveness to pKJK5 was highest and varied between 8.5×10^{-4} and 1.3×10^{-2} transfer per recipient, and that to RP4 was about one order of magnitude lower. Permissiveness to these two plasmids were correlated and was highest for the residential sewer samples. The cells that received the plasmids were sorted using flow cytometry and characterized by 16S rRNA gene amplicon sequencing. Preliminary analysis identified genera that are consistently capable of engaging in plasmid uptake at most points of the urban water cycle, highlighting their potential role as facilitators of antimicrobial resistance dissemination.

Microbiome and mobile antibiotic resistome in wastewater treatment plants and recycled wastewater products

Erica Donner^{*1}, Sotirios Vasileiadis², Gianluca Brunetti³, Jan Bell⁴, Xiayuan Wu³, Samuel Aleer³, Michael Short³, Christopher Saint³, Enzo Lombi³, Barbara Drigo³
¹*University of South Australia, Australia*, ²*Dept. Biochemistry & Biotechnology, University of Thessaly, Greece*, ³*Future Industries Institute, University of South Australia, Australia*, ⁴*The University of Adelaide, Australia*

Sanitation plays a key role in preventing the spread of infectious disease by concentrating a community's enteric microbial load into a single waste stream for treatment. However, wastewater treatment plants (WWTPs) are also highlighted as likely evolutionary hotspots for antibiotic resistance development. Current efforts to understand the complex microbial ecology and mobile antibiotic resistome in WWTPs will underpin their potential function as critical barriers mitigating the dissemination of resistant microorganisms and genes from humans and farm animals to downstream environments. Here, we used a suite of high-throughput molecular tools to monitor the structure and diversity of bacterial, fungal and protist communities, and antibiotic resistance gene dynamics in four major WWTPs. Methods included 16S/18S/ITS rDNA amplicon sequencing, shotgun metagenomics, qPCR and digital droplet PCR. Our extensive multi-season study focused not only on influents, effluents, and wastewater derived products (recycled water and biosolids) but also on the intermediate treatment stages and disinfection processes. Results demonstrate the efficacy of treatment stages such as ultra-filtration and lagoon storage for removing potentially harmful microbes from the water stream, but also reveal significant concentrations of antibiotic resistance genes in the sludge fractions destined for agricultural use and the potential for regrowth of resistant organisms in disinfected effluents. Many interesting trends have been observed. Dominant taxa of interest include *Arcobacter*, *Acinetobacter*, *Mycobacteria*, and *Streptococcus*; while qPCR results show that some key resistance genes (e.g. *blaKPC*) are commonly found in influents but not detectable in effluents, recycled water, and biosolids. Key network characteristics and hosts will be presented.

Culture independent determination of the host range of antibiotic resistance genes in wastewater treatment plants on single cell level

Marko Virta^{*}, Jenni Hultman, Katariina Pärnänen, Johannes Cairns, Manu Tamminen
University of Helsinki, Finland

Wastewater treatment plants (WWTPs) collect wastewater from various sources for a multi-step treatment process. By mixing a large variety of bacteria and promoting their proximity, WWTPs constitute potential hotspots for the emergence of antibiotic resistant bacteria.

Currently, studies on antibiotic resistance in WWTPs are based on quantitative PCR, metagenomics or cultivation. Because all of them have their specific drawbacks, linking of the linking taxonomic information to specific ARGs remains to be a challenge although it is essential for the understanding the ecology of ARGs and also for conducting risk assessment of the ARGs in the environment.

We utilized epicPCR (Emulsion, Paired Isolation and Concatenation PCR) to detect the bacterial hosts of antibiotic resistance genes in two WWTPs. We identified the host distribution of four resistance-associated genes (*tetM*, *int1*, *qacEΔ1*, *bla_{OXA-58}*) in influent and effluent. The bacterial hosts of these resistance genes varied between the WWTP influent and effluent, with a generally decreasing host range in the effluent. Through 16S rRNA gene sequencing it was determined that the resistance gene carrying bacteria include both abundant and rare taxa.

Our results suggest that the studied WWTPs mostly succeed in decreasing the host range of the resistance genes during the treatment process. Still, there were instances where effluent contained resistance genes in bacterial groups not carrying these genes in the influent. By permitting exhaustive profiling of resistance gene hosts in WWTP bacterial communities, the application of epicPCR provides a new level of precision to our resistance gene risk estimates.

Non-antibiotic pharmaceuticals promote horizontal transfer of multi-antibiotic resistance genes

Yue Wang^{*1}, Ji Lu², Zhiguo Yuan², Philip L. Bond², Jianhua Guo²

¹University of Queensland, Australia, ²the University of Queensland, Australia

The spread of antibiotic resistance represents a global threat to public health, killing at least 700,000 people worldwide annually. Horizontal gene transfer (HGT) is one of the most significant pathways to disseminate antibiotic resistance. It is commonly acknowledged that sub-minimum inhibition concentrations of antibiotics are major contributors in disseminating antibiotic resistance through HGT. Pharmaceuticals are occurring in our environments at increased levels, yet little is known whether non-antibiotic pharmaceuticals cause or accelerate the dissemination of antibiotic resistance. In this study, we aim to fill the critical knowledge gaps in antibiotic resistance induced by non-antibiotic pharmaceuticals. We chose a few commonly prescribed non-antibiotic pharmaceuticals (including antiepileptic drug and nonsteroidal anti-inflammatory drugs), to test whether they could promote conjugative transfer of multi-antibiotic resistance carried by RP4 plasmid within and across bacterial genera. Surprisingly, all of these pharmaceuticals with environmentally relevant concentrations can enhance conjugative transfer of antibiotic resistance genes significantly. The underlying mechanisms of the enhanced HGT were revealed by detecting oxidative stress and cell membrane permeability, in combination with MinION plasmid sequencing, genome-wide RNA sequencing and proteomics analysis. Non-antibiotic pharmaceuticals induced a series of acute responses, including over-producing reactive oxygen species, the SOS response; increasing cell membrane permeability, and pilus generation.

Expressional levels of core genes related to these processes significantly up-regulated due to non-antibiotic pharmaceutical exposure. Given that HGT occurs widely among different species in various environments, these findings are a wake-up call to start re-evaluating the roles of non-antibiotic pharmaceuticals in the spread of antibiotic resistance.

Effects of antibiotic treatment on fecal, ileal, and ileocecal lymph node-associated microbiota in pigs

Benjamin Zwirzitz^{*1}, Beate Pinior², Barbara Metzler-Zebeli³, Monika Handler¹, Kristina Gense¹, Monika Dzieciol¹, Christian Knecht⁴, Stefanie U. Wetzels¹, Andrea Ladinig⁴, Martin Wagner¹, Stephan Schmitz-Esser⁵, Evelyne Mann¹

¹University of Veterinary Medicine / Institute of Milk Hygiene, Austria, ²University of Veterinary Medicine / Institute for Veterinary Public Health, Austria, ³University of Veterinary Medicine / Institute of Animal Nutrition and Functional Plant Compounds, Austria, ⁴University of Veterinary Medicine / University Clinic for Swine, Austria, ⁵Iowa State University / Department of Animal Science, United States

In-feed antibiotics alter the gut microbiome and consecutively might also affect translocation processes of microorganisms to lymphatic tissues. As a result, intestinal microbiota and the host immune system could fall into an imbalanced state, making the host susceptible to recurrent infections and dysbiosis. Hence, understanding the variant effects of antibiotics on the microbiome in specific tissues is of vital importance for animal production and health. Here, we provide the first comparative study of microbial communities in pig feces, ileum, and ileocecal lymph nodes under the influence of in-feed antibiotics using 16S rRNA gene high-throughput sequencing. Furthermore, we also investigated the microbiome of ileocecal lymph nodes by cultivation, generating 95 isolates, and by sequencing the metatranscriptome of a single lymph node sample. The *Proteobacteria*-dominated lymph node microbiome represented a sub-fraction of the gut microbiome with a significant lower diversity compared with ileum and feces. In each analyzed tissue, we identified phylotypes susceptible to antibiotic treatment that hold profound impacts on the host physiological and immunological state, with the lymph node microbiome being affected by antibiotics to a lesser extent compared with feces and ileum. Pigs that received antibiotics harbored significantly reduced amounts of segmented filamentous bacteria along the ileal mucosa. RNA-sequencing of a lymph node unveiled expressed transcripts used for bacterial metabolic core processes like amino acid and carbohydrate metabolism, therefore proving the metabolic activity of bacteria in lymph nodes. Our results indicate that pathogenic bacteria could escape antibiotic treatment, if they are translocated to lymph nodes.

Polysaccharide-driven niche differentiation between distinct *Polaribacter* populations during North Sea spring algal blooms

Burak Avci*, Karen Krüger, Bernhard Fuchs, Hanno Teeling, Rudolf Amann
Max Planck Institute for Marine Microbiology, Germany

The remineralization of algal biomass by heterotrophic bacterioplankton during phytoplankton blooms is a globally important process in carbon cycling. We investigated North Sea spring phytoplankton blooms from 2009 to 2012 and observed a succession of distinct bacterioplankton clades. However, diversity and ecological niches of these bacterioplankton clades remained elusive. In this study, we explore the diversity and ecophysiology of *Polaribacter* spp. - the most abundant bacterioplankton clade during the studied algae blooms. We (i) identified distinct *Polaribacter* populations using phylogenetic analyses, (ii) quantified their abundances using fluorescence *in situ* hybridization, minimum entropy decomposition analysis and metagenome read recruitment and (iii) and retrieved largely complete *Polaribacter* genomes via metagenome binning. These evidences combined revealed a temporal succession of four major *Polaribacter* populations with varying polysaccharide utilization capacities. *Polaribacter* 2-a is a first responder with a small genome and limited polysaccharide utilization capability and likely features coupled protein and carbohydrate metabolisms. *Polaribacter* 3-a represents a secondary responder that reaches high abundances only in 2010 and has a distinct degradation potential for sulfated alpha-mannan. *Polaribacter* 3-b is a late responder with a pronounced sulfated xylan utilization capacity. *Polaribacter* 1-a has the largest genome and the most diverse polysaccharide degradation spectrum and is abundant after blooms of *Chattonella* algae. Our study suggests a polysaccharide-based niche separation between closely related bacteria during phytoplankton blooms and highlights how the release of diverse algal polysaccharides could create successive niche spaces in which distinct bacterioplankton clades thrive.

A mechanistic breakdown of carbon flow within the rumen microbiome

Live Hagen*¹, Adrian E. Naas¹, Angela D. Norbeck², Heather Brewer², Ljiljana Paša-Tolić², Vincent G. H. Eijsink¹, Magnus Ø. Arntzen¹, Matthias Hess³, Phillip B. Pope¹
¹*Department of Chemistry, Biotechnology and Food Science, NMBU - Norwegian University of Life Sciences, Norway*, ²*Environmental and Molecular Sciences Laboratory, Pacific Northwest National Laboratory, United States*, ³*Department of Animal Science, University of California, United States*

The rumen is an infamous plant fiber-digesting ecosystem, which harbors a complex mixture of bacteria, archaea, protozoa and fungi that coordinate breakdown of complex dietary carbohydrates into nutrients utilized by the animal. Despite extensive efforts to functionally map the rumen microbiome, cellulose degradation has so far been attributed to a limited number of cultivable representatives.

Moreover, the majority of the rumen microbiota, their complex interactions and the enzymatic machineries they employ remain poorly understood. Here, we present an overview of our recent efforts, where we combine traditional culturing, meta-omics, bioinformatics, biochemistry and enzymology to investigate the different saccharolytic mechanisms that rumen microbiota employ. We demonstrate key findings from studies on well-known *Fibrobacter succinogenes* isolates, a novel cellulolytic Bacteroidetes family ('*Candidatus* MH11'), and large-scale rumen meta-omics projects that seek to decrypt plant fiber metabolism and methane generation at a community level. This approach has revealed new mechanistic information related to the hydrolytic capacity of outer membrane vesicles (OMVs), Polysaccharide utilization loci (PULs) and large multi-modular enzymes secreted by *Ca. MH11*. In particular, (meta)genome-resolved metaproteomic data has generated deeper insights into the intricate networks of *in situ* plant fiber deconstruction. Importantly, these studies revealed a previously non-recognized role of the scarcely explored anaerobic rumen fungi. These results will be further discussed in the context of multiple large meta-omic datasets generated from rumen gut ecosystems.

Recovery of complete genomes from complex microbial communities using long-read Nanopore sequencing

Rasmus Hansen Kirkegaard*, Søren Michael Karst, Mads Albertsen
Aalborg University, Denmark

Short read DNA sequencing and metagenomic binning workflows have made it possible to extract bacterial genome bins from environmental microbial samples containing hundreds to thousands of different species. However, these genome bins often do not represent complete genomes, as they are mostly fragmented, incomplete and often contaminated with foreign DNA. The value of these 'draft genomes' have limited, lasting value to the scientific community, as gene synteny is broken and there is some uncertainty of what is missing. The genetic material most often missed is important multi-copy and/or conserved marker genes such as the 16S rRNA gene, as sequence micro-heterogeneity prevents assembly of these genes in the de novo assembly. However, long read DNA sequencing technologies are emerging promising an end to fragmented genome assemblies.

We extracted DNA from a full-scale anaerobic digester system and sequenced it with both short illumina based sequencing and long read Nanopore sequencing. This allows for direct comparison between the two and for hybrid assembly strategies benefitting from the strengths of both technologies.

We find that meta-genome assembly using Nanopore long reads is superior to short reads as the assemblies are much more contiguous and also include rRNA genes which often are an indicator of problems with strain diversity in complex samples. Furthermore, we find that it is even possible to make completely circular genome assemblies from complex full-scale samples with the throughput of the MinION DNA sequencer.

A multi-omic view of invasive genetic elements and their linked prokaryotic population dynamics within a mixed microbial community

Susana Martínez Arbas^{*1}, Shaman Narayanamsamy¹, Malte Herold¹, Laura Lebrun¹, Tony Lam², Nathan D. Hicks³, Lance B. Price³, John D. Gillece³, James M. Schupp³, Paul S. Keim³, Cedric Laczny¹, Yuzhen Ye², Karoline Faust⁴, Patrick May¹, Emilie E.L. Muller⁵, Paul Wilmes¹

¹University of Luxembourg, Luxembourg, ²University of Indiana, United States, ³The Translational Genomics Research Institute, United States, ⁴University of Leuven, Belgium, ⁵University of Strasbourg, France

Invasive mobile genetic elements (iMGEs), specifically (bacterio-)phages and plasmids, are highly abundant and diverse genetic components influencing the structure and dynamics of microbial communities. However, the roles of iMGEs are not well understood due to limitations in experimental and analytical methodologies. Fortunately, microbial community-derived high-throughput omic data, specifically metagenomics and metatranscriptomics, enable profound access to genomic and transcriptomic complements, including iMGEs. Here, we perform an integrated multi-omics study of iMGE-host dynamics within an extensively sampled (over 1.5 years) model system of lipid-accumulating microbial populations (LAMP) from a biological wastewater treatment plant. *De novo* co-assembly of metagenomic and metatranscriptomic data yielded 92 unique representative genomes (ReGes) traceable over time. 18 of these ReGes contained at least one complete CRISPR-Cas system; a memory-based prokaryotic defence system. Furthermore, we performed an extensive mining of CRISPR information, resulting in the identification of ~8,000 unique CRISPR-repeats and ~160,000 unique CRISPR-spacers, including their dynamics. We then inferred the putative iMGEs using computational phage and plasmid sequence prediction tools, as well as relying on CRISPR-spacer target information. Overall, ~42,000 unique phage and ~750,000 unique plasmid sequences were detected, while other iMGEs remained either ambiguous or unclassified. ~8,000 CRISPR spacer-repeat pairs were used to link 84 ReGes (hosts) to ~14,000 putative iMGE sequences. Finally, we highlight a dominant LAMP that actively utilizes the CRISPR-Cas system to target iMGEs, where we further describe the CRISPR-spacer gain and loss over time. In summary, our results suggest a dominance of the type-I CRISPR-Cas system and important interference against foreign plasmid sequences.

Colliding microbial communities to see what happens: Fecal microbiota transplantation experiments meet genome-resolved metagenomics

Andrea Watson^{*1}, Sonny T.M. Lee¹, Johanna Zaal DeLongchamp², Özcan C. Esen¹, Alon Shaiber¹, Bana Jabri¹, Thomas Louie², A. Murat Eren¹

¹University of Chicago, United States, ²University of Calgary, Canada

One of the fundamental pursuits of microbial ecology is to elucidate microbial interactions within complex ecosystems. This requires suitable experimental systems as well as effective computational strategies to expose naturally occurring microbial interactions at high resolutions. By effectively colliding two pre-established ecosystems from different individuals, fecal microbiota transplantation (FMT) experiments offer a dynamic system to gain insights into microbial succession and colonization. Here we exploited FMTs, and genome-resolved metagenomic strategies, to access the intricate within-population dynamics of human gut microbes. We deeply sequenced 119 fecal metagenomes from a time-series sampling of 2 donors, and 12 recipients before and after FMT. From these data we recovered more than 250 near-complete donor metagenome-assembled genomes (MAGs), which enabled us to track inter- and intra-population dynamics in recipient guts after FMT, and make multiple intriguing ecological observations. Among these observations were (1) competition events between highly-similar subpopulations distinguished by differential occurrence of mobile genetic elements within a single individual, and (2) differential colonization events between closely related taxa from the same donor into inflamed or non-inflamed recipients. Overall, our findings reveal the dynamic nature of complex gut ecosystems, and suggest a need for subpopulation-level characterizations of gut microbial ecology. The ability to work with novel population genomes not only links functional potential to observed phenotypes, but also gives appropriate targets for cultivation efforts to bridge the gap between 'omics studies and hypothesis testing for mechanistic insights.

Host genotype shapes the assembly of both the gut microbiota and the surrounding bacterioplankton in the freshwater crustacean *Daphnia magna*

Ellen Decaestecker^{*1}, Emilie Macke¹, Martijn Callens²
¹KU Leuven, Belgium, ²CNRS-CEFE Montpellier, France

The gut microbiota mediates important aspects of its host's biology, as well as tolerance to diverse environmental stressors. A key challenge is deciphering the factors dictating the assembly of this community, and establishing the relative contribution of evolutionary and ecological processes to the intraspecific variation frequently observed in the gut microbiota structure. Combining metagenetics with microbiota transplants, we here show that in the freshwater crustacean *Daphnia magna*, host genotype and diet interact to shape the structure of both the gut microbiota and the surrounding bacterioplankton. When different *Daphnia* genotypes were exposed to identical microbial communities, both the gut microbiota and the bacterioplankton diverged to reach a genotype- and diet-dependent taxonomic composition. The exposure of germ-free *Daphnia* to different microbial inocula also revealed an effect of the external microbial source on the gut microbiota structure. Overall, the taxonomic composition of the gut microbiota was however very different from that of the bacterioplankton, and was characterized by a lower alpha diversity, suggesting a selective, genotype-dependent, recruitment of gut symbionts in this species. Together, our results indicate strong reciprocal interactions between *Daphnia*, their gut microbiota and the bacterioplankton. Importantly, we provide evidence that *Daphnia* mediate the assembly of their associated microbial communities, both within their gut and in their close environment, depending on their genetic background. This result clearly demonstrates the impact of evolution (i.e. genetics) on ecological processes (i.e. community assembly) and, by illustrating an evo-to-eco link, provides strong support to eco-evolutionary dynamics theory.

The emergence of microbial community variability in similar environments

Sylvie Estrela^{*}, Nanxi Lu, Alvaro Sanchez
Yale University, United States

Microbes live in highly complex and diverse communities, but how such communities form, develop, and are maintained is not well understood. Of particular note is the role of historical contingency, and how it affects the phylogenetic composition of stable communities. Starting from a single source soil microbiome, we show that multiple alternative community states can arise from replicate communities propagated under identical environmental conditions. We visualize the development of these communities through time using a combination of community phenotyping and genotyping. These alternative community states are not only different structurally (species composition) but also exhibit different dynamical properties, including community growth rate, temporal stability, and synchrony. Furthermore, we find that such variability can be reduced either by increasing the size of the founder population or by immigration. Together, our results suggest that

community assembly history can lead to both structural and functional variability, even in simple and identical environments.

Predation and competition determine the stability of cooperation in bacteria

Zhilei Gao*, George Kowalchuk, Alexandre Jousset
Utrecht University, Netherlands

The production of toxic secondary metabolites by soil microbes is an essential component of competitiveness. Secondary metabolites are a public good and, as such, potentially vulnerable to cheating. Here, we address how competition and predation shape the benefits and stability of cooperation in relation to antibiotic production. We compare the fitness of cooperative *Pseudomonas fluorescens* and a selfish *gacS* isogenic mutant lacking the ability to produce these secondary metabolites. We grew the bacteria along a resource availability gradient in the presence of competitors (background microbial community) and predators (protists). We found that resource competition was the main driver of the benefit of cooperative behaviors. In contrast, predation was a highly stabilizing force, preventing the spread of defectors. Together, these results indicate that biotic interactions are essential to promote the persistence of antibiotics production in the rhizosphere microbiome, a property that is critical to the suppression of plant diseases.

Microbial communities vary in their carbon cycling response to climate

Sydney Glassman*¹, Claudia Weihe², Adam C. Martiny², Kathleen K. Treseder², Steven D. Allison², Jennifer BH Martiny²
¹*UC Riverside, United States*, ²*University of California, Irvine, United States*

Bacteria and fungi drive decomposition, a fundamental process in the carbon cycle, yet the importance of microbial community composition for decomposition remains elusive.

Here, we used an 18-month reciprocal transplant experiment along a climate gradient in Southern California, USA to disentangle the effects of the microbial community versus the environment on decomposition. We used “microbial cages” that prevent microbial exchange with the environment to inoculate microbial communities from each site onto a common, irradiated leaf litter. We then characterized fungal and bacterial composition and abundance over time and investigated the functional consequences by measuring litter mass loss and litter chemistry.

After transplantation, fungal communities retained a strong signature of microbial inoculum, even after 18 months ($R^2 = 0.41$, $P < 0.01$), whereas bacterial communities converged according to local climate ($R^2 = 0.47$ $P < 0.01$). However, a

strong site by inoculum interaction effect for both fungi ($R^2 = 0.18$, $P < 0.01$) and bacteria ($R^2 = 0.14$, $P < 0.01$) indicated that not all communities responded similarly to the climate gradient. Interestingly, microbial communities from the intermediate sites had significantly different impacts on decomposition and types of carbon compounds degraded when transplanted. Moreover, significant site by inoculum interactions impacting decomposition lasted a year after transplantation.

Not only does decomposition depend on microbial community composition, but the functional response of fungal and bacterial communities to climate varies. Thus, in order to accurately predict how ecosystems will respond to climate change, we must consider microbial composition.

Role of co-evolution in eco-evolutionary community dynamics

Teppo Hiltunen^{*1}, Lutz Becks², Johannes Cairns³

¹University of Helsinki, Finland, ²MPI, Germany, ³U. Helsinki, Finland

Understanding when and how rapid evolution drives ecological change is fundamental for our understanding of almost all ecological and evolutionary processes such as community assembly, diversification and the stability of communities and ecosystems. Evolution has been recognized to significantly alter especially the interaction between consumers and their resources, a key interaction in all ecological communities. While these eco-evolutionary dynamics have been shown to occur when prey populations are evolving, little is known about the role of predator evolution and co-evolution between predator and prey in this context. Here I present results from a series of experiments on how predator co-evolution affects the link between rapid evolution and ecological change using experimental evolution with the bacterium *Pseudomonas fluorescens* and its predator *Tetrahymena thermophila*. Three years ago, inspired by Richard Lenski's famous long-term evolutionary experiment, we started selection lines where eight different bacterial species evolve alone and with the ciliate *Tetrahymena thermophila* in pairwise combinations (each bacterium with *T. thermophila*). With this system, we have created a massive "fossil record" of co-evolving communities over thousands of generations. In addition to presenting the first results from this long-term experiment, I will present results from two additional experiments on how co-evolution increases the synchrony and stability of communities and functions as a driver of bacterial species coexistence.

Artificial selection of rhizosphere microbiota associated to phenotypical changes in plant functions

Samuel Jacquiod^{*1}, Aymé Spor¹, Søren Sørensen², Laurent Philippot¹, Manuel Blouin¹

¹Agroécologie, AgroSup Dijon, INRA, Univ. Bourgogne Franche-Comté, France, ²Section of Microbiology, University of Copenhagen, Denmark

Artificial selection applied at community level is an important, but still growing topic at the interface of ecology and evolution. Its recent implementation to microbial communities holds appealing promises not only in terms of fundamental knowledge about selection itself and the levels at which it may be used, but also in terms of relevant applications to our societies, including bioremediation and agroecology.

In this experimental evolution study, we performed an artificial selection of rhizosphere microbial communities inducing relevant phenotypic changes in plants. In total, we grew more than 2200 *Brachypodium distachyon* plants, consisting in ten consecutive generations of four-weeks, and inoculated with artificially selected rhizosphere microbiota originating from the previous generation. Selection was applied for increasing or decreasing plant nitrogen uptake based on leaves color nuances via a semi-automated high-throughput plant phenotyping platform. Ultimately, selected rhizosphere microbiota were also inoculated to maize, barley and wheat grown in two different soils to evaluate the transferability of our induced microbial properties.

16S rRNA gene and ITS amplicon sequencing revealed a rapid response of plants to artificial rhizosphere microbiota selection, with significant divergence and heritability of traits observed after few generations, correlating with specific changes in the structure of selected microbial pools. Transferability assay showed that properties linked to selected microbial communities depend on plant species, but consistently induced reproducible effects on *Brachypodium distachyon* in different soils. Artificial selection of rhizosphere microbiota altering plant phenotype is efficient, fast and reproducible, leveraging a whole new array of possibilities for shaping plant traits.

Spatial chaos: Can we predict patterns of spatial self-organization within microbial communities?

David Johnson*, Felix Goldschmidt, Lea Caduff
Eawag, Switzerland

Spatially structured microbial communities comprise a vast amount of microbial life on our planet. These microbial communities typically consist of different species or strains that interact with each other and arrange themselves in space non-randomly (referred to as spatial self-organization). While spatial self-organization can have important effects on community-level properties, the underlying factors causing spatial self-organization remain unclear. Using a synthetic cross-feeding microbial community consisting of two isogenic mutant strains of the bacterium *Pseudomonas stutzeri*, we demonstrate that two fundamentally different patterns of spatial-self organization can emerge simultaneously as the microbial community expands into unoccupied space. The simultaneous emergence of the different patterns is not caused by spatial heterogeneity in the initial abiotic environment or by genetic heterogeneity within populations. Instead, it is caused by non-genetic heterogeneity within populations. More specifically, we demonstrate that the two different patterns

likely emerged as a consequence of spatial chaos, where variation in the initial spatial positioning of individual cells gave rise to the multiple patterns of spatial self-organization. We further demonstrate that spatial self-organization has important effects on community-level properties; namely, the speed at which the microbial community expands into unoccupied space. Together, our results demonstrate that a single metabolic interaction can simultaneously give rise to fundamentally different patterns of spatial self-organization, and that spatial self-organization can have important effects on community-level properties. Moreover, our results raise general caution about using pattern analysis alone to infer interactions between species or strains.

Plasticity of endospheric microbiome

Minna-Maarit Kytöviita*, Anbu Poosakkannu
Jyväskylä University, Finland

Adaptation by phenotypic plasticity is classically studied in plants. Reciprocal transplantation of plants between different habitats is a method to evaluate plasticity in terms of endospheric microbiome. Plasticity can be measured as the extent the microbiome is altered by a change in environment. We transferred clonal fragments of the grass *Deschampsia flexuosa* between two contrasting habitats (exposed sand, forest) in subarctic sand dunes and measured the plant performance and endospheric microbiome change after 3 years. Transferring plants from their local environment into a markedly different one results in mismatch between the endospheric microbial community and the environment. This may result in (i) original endosphere microbial community is displaced by destination habitat microbes resulting in gain and loss of microbial taxa and shifts in relative abundance and (ii) plant performance is lower in the novel habitat if the original endophytic community lingers in novel environment.

Plant performance in the novel habitat and bacterial microbiome plasticity in roots and leaves correlated positively in the transplanted grass. This suggests that recruited destination habitat bacteria were important components of plant performance. Bacteria and fungi in leaf and root endosphere compartments responded differently to the reciprocal transplantation. In contrast to bacteria, the root fungal endophytic microbiome lingered in plants transplanted from forest to exposed sand corresponding with the lower plant performance. Altogether, the endospheric microbiome is plastic, but the adaptation to novel environmental conditions is a slow process. Transplantation studies are useful in evaluating the plasticity of microbiome and possible functional response to novel environment.

Cohesiveness in microbial community coalescence

Nanxi Lu^{*1}, Alicia Sanchez-Gorostiaga¹, Mikhail Tikhonov², Alvaro Sanchez¹
¹*Yale University, United States*, ²*Stanford University/Washington University, United States*

Microbial invasions exhibit many unique properties; notably, entire microbial communities often invade one another, a phenomenon known as community coalescence. In spite of the potential importance of this process for the dynamics and stability of microbial community assembly, our understanding of it is still very limited. Recent theoretical and empirical work has proposed that large microbial communities may exhibit an emergent cohesiveness, as a result of collective consumer-resource interactions and metabolic feedbacks between microbial metabolism and the environment. A fundamental prediction of this theory is the presence of ecological co-selection during community coalescence, where the invasion success of a given taxon is determined by its community members. To establish the generality of this prediction in experimental microbial communities, we have performed over one hundred invasion and coalescence experiments with environmental communities of different origins that had spontaneously and stably assembled in two different synthetic aerobic environments. We show that the dominant species of the coalesced communities can both recruit their community members (top-down co-selection) and be recruited by them (bottom-up co-selection) into the coalesced communities. Our results provide direct evidence that collective invasions generically produce ecological co-selection of interacting species, emphasizing the importance of community-level interactions during microbial community assembly.

ColE1 plasmids potentiates the fitness of *Salmonella* Heidelberg in poultry litter

Adelumola Oladeinde^{*1}, Kimberly Cook², Alex Orlek³, Greg Zock², Nelson Cox², Kyler Herrington⁴, Jodie Plumblee², Carolina Hall²

¹*United States Department of Agriculture, Agriculture Research Service, Athens, Georgia, United States*, ²*USDA-ARS, United States*, ³*Nuffield Department of Medicine, John Radcliffe Hospital, University of Oxford, Oxford,, United Kingdom*, ⁴*University of Georgia, United States*

Salmonella enterica subsp. *enterica* serovar Heidelberg (S. Heidelberg) is a clinically-important serovar, linked to food-borne illness, and commonly isolated from poultry. Investigations of a large, multistate outbreak in the USA in 2013 identified poultry litter as an important extra-intestinal environment that may have selected for specific S. Heidelberg strains. Poultry litter (PL) is a mixture of bedding materials and chicken excreta that contains chicken gastrointestinal (GI) bacteria, undigested feed, feathers, and other materials of host origin. In this study, we performed a series of controlled laboratory experiments which assessed the evolution of two S. Heidelberg strains (SH-2813 and SH-116) in PL previously used to raise 3 flocks of

broiler chickens. The strains are closely related at the chromosome level, differing by only 69 single-nucleotide variants. Whole genome sequencing was performed on 86 isolates recovered after 0, 1, 7 and 14 days of evolution in PL. Only strains carrying an IncX1 (37kb), 2 ColE1 (4 and 6kb) and 1 ColpVC (2kb) plasmid survived past 7 days in PL. Competition experiments showed that carriage of these plasmids was associated with increased overall fitness. This increased fitness was associated with an increased copy number of IncX1 and ColE1 plasmids. Additionally, we observed a decrease in susceptibility to tobramycin, kanamycin, gentamicin, neomycin and fosfomycin for Col plasmid-bearing strains. Our study demonstrates how acquisition of multiple Col-like plasmids can change the evolutionary path of *S. Heidelberg* in poultry litter.

A drop in a bucket makes a difference: enriching with pre-evolved community boosts function and shapes the structure of methane-producing communities

Pawel Sierocinski*, Angus Buckling
University of Exeter, United Kingdom

The structure and function of complex microbial communities are shaped by an interplay of ecological and evolutionary forces. Most research on evolutionary dynamics deals with simple systems and research on complex systems looks mainly at the ecological change. It is not clear, however, how short-term evolution impacts a multi-species community where all components evolve simultaneously.

To test it, we used methane-producing communities, where methane production can be used as a proxy for community function. To limit the impact of the ecological processes we pre-adapted replicate communities to a lab-scale fermenter system and transferred 1% of the pre-adapted community to the ancestral one. We compared the performance of the 1%-augmented communities with a non-augmented control. This did not impact the initial species composition, but instead enriched the communities with alleles favoured by selection.

Augmented communities produced significantly more methane than both the ancestral community and non-enriched control. They also contained species, detectable neither in the source we used to enrich them, nor in the control treatment. These data suggest that rapid evolution plays a crucial role in shaping the microbial community structure during the adaptation to a novel environment. It also impacts the functioning of communities in a highly reproducible way: the pattern was consistent across twelve replicates. Our results suggest that pre-adaptation strategies are potentially a valuable tool for optimising biotechnological processes that rely on complex microbial communities.

Climate change effect in organic matter decay and ecological succession in mangroves

Juanita Solano¹, Rodrigo Taketani^{*2}

¹*Embrapa Environment, Brazil*, ²*University of São Paulo, Brazil*

Mangrove are coastal environments that provide resources for adjacent ecosystems due to its high productivity that comes from decay of organic matter and carbon cycling. Based on the hypothesis that climate change modifies microbial diversity associate to decay of organic matter in mangrove sediments, changing the emission of Greenhouse Gases rate, the goal of this research is to evaluate the dynamics of microbial diversity under the climate change conditions during de decay process, correlating with the emission of GHG. Destructive microcosms containing organic matter from the main plant species found in Brazilian mangroves (*Rhizophora mangle*, *Laguncularia racemosa* and *Avicennia schaueriana*) were incubate simulating climate changes (increase in temperature and pH). The variation in time resulted in important increases of alpha diversity impacts and in the community composition, initially with greater abundancy of Gammaproteobacteria for all plant species despite of the climate conditions variations. The PCoA analysis shows the chronological sequence in β diversity, indicating the increase of Deltaproteobacteria at the end of the process. The GHG emission varied in function of the organic matter source and the correlation between methane (CH₄) release and the presence of the mcrA gene in two of the plant species studied, if the increase in the Deltaproteobacteria population controlled its emission. Despite the great number of studies about the decay of organic matter and emission of gases in mangroves, few present an approach like this work, which aims to understand the relation between these three processes and the climate changes, a pressing problem nowadays.

Understand microbial biodiversity through the lens of field experiments along climatic zones

Jianjun Wang^{*1}, Feiyan Pan², Janne Soininon³, Jani Heino⁴, Ji Shen¹

¹*Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences, China*, ²*Nanjing Normal University, China*, ³*University of Helsinki, Finland*, ⁴*Finnish Environment Institute, Biodiversity Centre, Finland*

Spatial patterns in biodiversity are one of the core topics in ecology; however, the mechanisms driving these patterns remain unclear. Climatic factors, especially temperature, are regarded as the main drivers underlying diversity gradients at broad spatial scales. On the other hand, human impacts, such as nutrient enrichment, have been identified as one of the main drivers of biodiversity loss in recent decades. A promising approach to explore climatic effects would be macroecological experiments (i.e., broad-scale field experiments) on mountainsides. This approach integrates elevational gradients with experimental manipulations of nutrient enrichment to explore the independent effects of climate and human

impacts on biodiversity. Here, we conducted comparative field experiments, paralleled in subarctic and subtropical regions, to examine the independent effects of temperature and nutrient enrichment on aquatic bacterial biodiversity and community composition from the taxonomic, functional and phylogenetic perspectives. Bacterial communities allow us to examine diversity patterns in natural field conditions subject to real species pool effects, which cannot be conducted in laboratory conditions, or for macroorganisms, within feasible time periods. For instance, from the taxonomic perspective, temperature plays a pivotal role in maintaining elevational biodiversity patterns but its effects are modified by nutrient enrichment such that temperature effect on richness is strongest at very low or high nutrient levels. The findings offer examples of the importance of the study of global changes using integrating experiments and natural environmental gradients, and illustrate an approach which can be distributed globally to advance our predictive understanding of ecological trends and responses.

Evolution of phage resistance and virulence of *Pseudomonas aeruginosa* in a microbial community context

Edze Westra*, Ellinor Alseth, Ellie Pursey
University of Exeter, United Kingdom

Pseudomonas aeruginosa is an opportunistic pathogen known for its ability to rapidly evolve antibiotics resistance, and is as such increasingly becoming a target of clinical phage therapy trials. However, the rapid evolution of phage resistance mechanisms remains an issue, with approximately half of all *P. aeruginosa* clinical isolates possessing adaptive CRISPR-Cas immune systems to combat viral infection. While the widespread nature of CRISPR systems suggests they are important in clinical settings, lab-based studies often find that *P. aeruginosa* almost exclusively evolves surface-based resistance. This discrepancy may be explained by differences in the biotic and abiotic environment between *in vitro* and *in vivo* environments. In particular, while *P. aeruginosa* is typically examined in isolation when grown in the lab, during clinical infections *P. aeruginosa* usually coexists with a polymicrobial community of other pathogens. Here, we report how an artificial cystic fibrosis microbial community, consisting of *Staphylococcus aureus*, *Burkholderia cepacia* complex and *Acinetobacter baumannii*, drives the evolution of CRISPR-based phage resistance in *P. aeruginosa* PA14. Using a *Galleria mellonella* infection model, we also show that the evolution of CRISPR-based resistance results in the maintenance of virulence on par with the ancestral, while the evolution of surface-based resistance leads to reduced virulence *in vivo*. Collectively, our analyses demonstrate how intra-host biodiversity might propagate the evolution of CRISPR resistance in clinical settings, and that the type of resistance mechanism evolved has important implications for *P. aeruginosa* virulence.

The impact of an active microbial community on N₂O, CH₄, and CO₂ flux in Arctic mineral cryosols

Ianina Altshuler^{*1}, Elisse Magnuson², Charles Greer³, Lyle Whyte²

¹McGill University (Macdonald Campus), Canada, ²McGill

University, Canada, ³National Research Council, Canada

Climate warming and subsequent permafrost thaw allows the currently frozen carbon and nutrient stores to become available for metabolism by microbial communities. This can result in a positive feedback loop of greenhouse gas (GHG) soil emissions. Nitrous oxide (N₂O) methane (CH₄), and carbon dioxide (CO₂) are the most important GHGs. Through a combination of Metatranscriptomics, qPCR, gas flux, functional gene sequencing, and *in situ* stable isotope probing (SIP) we demonstrate an active community, likely responsible for positive N₂O and CO₂ fluxes and a negative CH₄ flux at a High Arctic cryosol site. We report a higher abundance of denitrification and methanotrophy genes in the soils exhibiting higher N₂O emissions and higher CH₄ uptake. These genes were also detected in the soil metatranscriptomes, indicating an active community of denitrifiers and methane oxidizers present in the soils. Functional gene sequencing of *nirS* and *nifH* genes reflected the differences in the microbial community with higher N₂O flux, these soils contained higher abundance of Rhodocyclales, Desulfobacterales, and Gallionellales orders. Finally, *in situ* SIP with C¹³ methane and subsequent metagenome and *pmoA* sequencing demonstrated that all the labelled *pmoA* genes were related to high-affinity methanotrophs, suggesting this group of organisms is responsible for the CH₄ sink. The active microbial community in the mineral soils is likely responsible for apparent N₂O and CO₂ emissions and CH₄ uptake in the High Arctic. The differences between gas fluxes are mirrored in the dominant microbial community members and the abundance of relevant functional genes in these soils.

Molybdenum availability controls vanadium nitrogenase activity in boreal forest cyanolichens

Romain Darnajoux^{*1}, Nicolas Magain², Marie Renaudin³, Jolanta Miadlikovska², François Lutzoni², Xinning Zhang¹, Jean-Philippe Bellenger³

¹Princeton University, United States, ²Duke University, United States, ³Université de Sherbrooke, Canada

Cyanobacteria associated with mosses and lichens are important contributors to the nitrogen cycle in high latitude ecosystems. They can synthesize nitrogenase metalloenzymes that catalyze the reduction of atmospheric dinitrogen into bioavailable ammonia. Besides the canonical molybdenum nitrogenase, present in all nitrogen fixers, two “alternative” isozymes have been reported: the vanadium dependent and the iron-only dependent nitrogenases. Because the molybdenum nitrogenase has long been thought to be the only nitrogenase isozyme with ecological relevance, the role of alternative nitrogenases in natural habitats has yet to be explored.

Here, we show evidence for a wide-spread activity of alternative nitrogenases in the cyanobacteria *Nostoc* associate with *Peltigera* lichens. We collected samples across a 1300 km long south-north transect crossing the Canadian boreal biome, and over the entire growing season (May to October). Using state-of-the-art genetic and metabolic characterization methods, we found the presence of the genes and signatures of the use of the vanadium nitrogenase. We further show that the low availability of molybdenum in boreal habitats is the most likely cause of vanadium nitrogenase activity in the *Nostoc* of these lichens.

The results demonstrate the ecosystem-wide importance of a previously overlooked enzyme for biological nitrogen fixation and call for a re-evaluation of the importance of alternative nitrogenases in natural habitats. They also bring new insights on the biogeochemical coupling of the cycles of micronutrient trace metals with those of major elements. This is particularly relevant because of the increasing body of studies reporting molybdenum limitation of dinitrogen fixation in many ecosystems.

It's getting hot in here: impacts of increasing temperature on the cycling of carbon and nitrogen in pristine Arctic streams

Sarah Faye Harpenslager^{*1}, Kate Randall², Michelle Jackson³, Bruno Gallo³, Danielle Harris², Hannah Prentice², Guy Woodward³, Alex Dumbrell², Mark Trimmer¹

¹Queen Mary University of London, United Kingdom, ²University of Essex, United Kingdom, ³Imperial College, United Kingdom

With global temperatures expected to rise in coming decades, understanding how the major biogeochemical cycles respond to warming is key to predicting how global climate change will affect cycling of energy - i.e. important elements such as carbon and nitrogen - through ecosystems. Currently, predictions are often derived from eutrophic environments and may therefore not be valid for pristine regions like the Arctic, where global warming is expected to have severe impact.

In 5 different Arctic locations, we used stable isotopes to measure rates of potential denitrification, nitrification, methane oxidation and methanogenesis in streams along a natural temperature gradient. Furthermore, we determined warming effects on the associated microbial community by analysing abundance of key genes and on whole ecosystem metabolism by diel oxygen measurements.

Our initial results show that in these oligotrophic Arctic streams, warming only increases microbial activity when nutrients are relatively abundant. In highly oligotrophic environments, however, extremely low nitrogen and phosphorus availability limited activity to such a degree that temperature effects became negligible. This is supported by much lower overall microbial biomass and lower functional gene abundance in low nutrient systems.

By combining biogeochemical and molecular approaches, we found that enhanced microbial activity by warming might be partially counteracted by low nutrient availability in pristine natural systems. Nutrient availability may thus be an important

factor that needs to be included to improve validity of predictive models used to assess the effects of global warming on the functioning of ecosystems.

Life in Pleistocene permafrost: A tale of survival strategies and carbon metabolism

Rachel Mackelprang

California State University Northridge, United States

One quarter of the earth's terrestrial surface is underlain by permafrost, or perennially frozen soils. Permafrost soils contain approximately 25-50% of the total global soil C pool, which is largely protected from microbial decomposition by frozen conditions. However, climate change is threatening to induce large-scale permafrost thaw exposing it to degradation. The resulting production of greenhouse gasses is expected to result in a positive feedback loop amplifying the effects of global warming. Carbon degradation in response to thaw will likely depend on multiple factors including physicochemistry, microbial community processes, and age. In this study, we compared how permafrost age, history, and chemistry drives the ability of microbial communities to degrade carbon. We combined metagenomic sequencing of microbial communities and FTICR-MS analysis across a Pleistocene permafrost chronosequence from 19,000 to 33,000 years before present (kyr).

We found that microbial communities adapt to life in permafrost through geologic time as evidenced by an increasing abundance of genes involved in the survival under frozen static conditions. However, the ability to degrade carbon was not influenced by age. Instead, carbon processing genes were correlated with carbon composition, vegetation present at the time the permafrost formed, and paleoclimate. Here, we describe how carbon processing capabilities are related to carbon chemistry and the paleoenvironment. The ultimate fate of carbon from permafrost depends on the complex relationship between permafrost physiochemistry and microbial communities. Therefore, understanding how the two interact will be important for predicting greenhouse gas emissions from the thawing permafrost.

Copepod-associated anaerobic nitrogen cycling across ecosystems and taxonomic groups

Peter Stief^{*1}, Ann Sofie B. Lundgaard¹, Hans Peter Grossart², Torkel G. Nielsen³, Alvaro Morales Ramirez⁴, Bo Thamdrup¹, Ronnie N. Glud¹

¹University of Southern Denmark, Denmark, ²Institute of Freshwater Ecology and Inland Fisheries, Germany, ³Technical University of Denmark, Denmark, ⁴Universidad de Costa Rica, Costa Rica

Organic aggregates sinking through marine and freshwater water bodies are increasingly perceived as abundant hotspots of anaerobic microbial metabolism that cannot take place in the surrounding oxygenated water. While the research focus is often on phytoplankton-derived aggregates, zooplankton may also provide anoxic microenvironments, such as guts, fecal pellets, and carcasses of copepods. We tested therefore, if anaerobic nitrogen cycling is associated with copepods collected from polar, temperate, and tropical marine or freshwater ecosystems and belonging to different taxonomic groups and size classes. Dissimilatory nitrogen transformations were quantified using ^{15}N tracing with living and dead copepods and with fecal pellets at different ambient oxygen levels to mimic conditions prevailing in the respective ecosystem. Significant anaerobic nitrogen cycling was without exception associated with copepod carcasses and was also evident in living specimens and fecal pellets of *Calanus hyperboreus*, a key component of Arctic marine zooplankton. In this large copepod species, denitrification was the dominant pathway of anaerobic nitrogen cycling and correlated with the presence or absence of food particles in the gut, but not with ambient oxygen levels. In contrast, the carcasses of the much smaller tropical marine and temperate freshwater copepods displayed high rates of dissimilatory nitrate reduction to ammonium or nitrite that were boosted by low ambient oxygen levels. Extrapolated to their *in situ* carcass abundance, copepods contribute up to 28% to the total fixed-nitrogen loss in oxic/hypoxic settings and up to 14% in anoxic settings. Overall, copepods greatly expand the water volume in which anaerobic nitrogen cycling is possible.

Assimilation and dissimilation of sulfate by anaerobic methane-oxidizing consortia

Hang Yu^{*1}, Connor Skennerton¹, Grayson Chadwick¹, Andy Leu², Roland Hatzepichler¹, Danielle Goudeau³, Rex Malmstrom³, Tanja Woyke³, Gene Tyson², Victoria Orphan¹

¹California Institute of Technology, United States, ²University of Queensland, Australia, ³Joint Genome Institute, United States

Teragrams of methane in marine ecosystems are consumed by consortia of syntrophic anaerobic methane-oxidizing archaea (ANME) and sulfate-reducing bacteria (SRB), yet the basis of their syntrophy remains poorly understood. Here we used activity-based fluorescence activated cell sorting to obtain single-consortium genomes between specific subgroups of ANME and SRB. We then leveraged these genomes in a metatranscriptomic investigation of how particular ANME-SRB partnerships respond in the presence of an artificial electron acceptor instead of the natural electron acceptor sulfate. The overall expression profiles indicated that SRB were transcriptionally inactive with the artificial electron acceptor, likely due to a lack of methane-derived electrons from ANME. These metatranscriptomic observations paralleled the single-cell anabolic activity patterns observed with $^{15}\text{NH}_4^+$ stable isotope probing using nanoscale secondary ion mass spectrometry. In the presence of both artificial electron acceptor and sulfate, ANME carried out methane oxidation alone at faster metabolic rates, with higher transcriptional and anabolic activities. However, sulfate was not significantly consumed and all known dissimilatory sulfate reduction genes were still transcriptionally inactive, suggesting that pathways of

methane oxidation and dissimilatory sulfate reduction are not linked in one organism as proposed previously. It is possible that ANME assimilate a small amount of sulfate based on our genomics and transcriptomics results and suggest an underappreciated assimilatory role of sulfate in anaerobic methane metabolism. By combining these cellular activity analyses, our results provide insight into the syntrophic interaction between ANME and SRB that link the cycling of carbon and sulfur together.

Microbial metabolism shift to hydrocarbon degradation in the deepest seawater on earth

Xiao-Hua Zhang^{*1}, Jiwen Liu¹, Yanfen Zheng¹, Heyu Lin¹, Xuchen Wang¹, Meng Li², Meixun Zhao¹, Nikolai Pedentchouk³, David Lea-Smith³, Jonathan Todd³, Jiwei Tian¹, Xiao-Hua Zhang¹

¹*Ocean University of China, China*, ²*Shenzhen University, China*, ³*University of East Anglia, United Kingdom*

The Mariana Trench is the deepest place on Earth, reaching a depth of ~11,000 m at the Challenger Deep. Its hadal waters (>6,000 m) constitute 45% of the vertical depth gradient. Recent studies demonstrate that hadal waters harbor distinct microbial planktonic communities. However, the ecological capacity of microbial communities within the hadal zone are poorly understood. Here we show an abrupt increase in the relative abundance of hydrocarbon-degrading bacteria from waters at 9,600 to >10,000 m in the Challenger deep of the Mariana Trench. These bacteria were represented mainly by *Oleibacter*, *Thalassolituus* and *Alcanivorax* (~25% of the metagenome), all of which include species that can consume aliphatic hydrocarbons. This community shift towards hydrocarbon degraders was accompanied by an enrichment for genes involved in alkane degradation. Correspondingly, two *Alcanivorax* species that were isolated from 10,400 m, in addition to a reference *Oleibacter* strain, were able to efficiently degrade a wide range (C₁₁-C₃₆) of *n*-alkanes. *n*-alkanes (dominated by medium-chain lengths of C₁₅-C₂₃), derived from complex sources, were detected in both the sinking particles and surface sediment (~10,910 m), suggesting that these compounds support this hydrocarbon-degrading bacterial population. Overall, these results reveal an unexpected and unique biosphere dominated by hydrocarbon catabolism in the deepest seawater on earth, shedding new light on biological processes in extreme environments.

Probing the soil microbial interactome

Manupriyam Dubey*, Noushin Hadadi, Jan Roelof van der Meer
University of Lausanne, Switzerland

Microbial communities are a crucial part of healthy soil ecosystems, but may become seriously perturbed in polluted sites. One possibility for potential community restoration is to introduce one or more species with specific capacities, for example, to detoxify the pollutants. However, the ability of an exogenous inoculant to thrive in any particular existing microbial community is often unpredictable, and subject to a plethora of biotic and abiotic factors.

This work aims towards predicting the success of strain inoculation from massive parallelized co-cultivation with soil community members inside agarose microbeads. As a proof of concept, we produce random pair-wise combinations of soil community members and *Pseudomonas veronii* 1YdBTEX, a toluene degrader, or *Pseudomonas protegens* and *Escherichia coli* as controls. The beads act as growth chambers for embedded cells and are suspended in specific (toluene) or general carbon substrate medium. Growth is characterized by epifluorescence microscopy at regular time intervals, and microcolony sizes of partners within beads are taken to infer positive or negative interactions (the "interactome"). Results suggest soil community members to profit from being together with an inoculant irrespective of carbon substrate used, in comparison to absence of inoculant. *Pseudomonas* strains generally fare better with soil community members than *E. coli*. Our interactome method by encapsulation to determine pair-wise growth interactions may be more generally applicable for testing community networks.

Biofilm thickness controls the contribution of stochastic and deterministic processes in microbial community assembly

Susan Jane Fowler*¹, Elena Torresi², Arnaud Dechesne¹, Vaibhav Diwan¹, Magnus Christensson², Barth Smets¹

¹Technical University of Denmark, Denmark, ²Veolia Water Technologies AB, AnoxKaldnes, Sweden

Niche and neutral theories provide diverging viewpoints on the importance of selection and neutral processes in community assembly. In practice, both deterministic and stochastic factors play a role in microbial community assembly, though little is known about manipulating their relative importance. We investigated the effect of biofilm thickness on community assembly using Z-carriers[®], biofilm carriers where biofilm thickness is controlled by grid height. Duplicate Z-carriers of five thicknesses (50-500 µm), influent and effluent were sampled at intervals from steady-state nitrifying reactors. Extracted DNA was subjected to 16S rRNA amplicon sequencing and qPCR for Bacteria. The biofilm communities were distinct from influent and effluent communities and exhibited greater temporal stability which increased with thickness. Biofilm communities were strongly influenced by selection as few sequence variants (SVs) were shared between the carriers and influent, however, the number of shared SVs increased with biofilm thickness. Neutral

modelling revealed that a greater percentage of shared SVs were neutrally assembled with increasing thickness, corresponding to a linear relationship between biofilm thickness and migration rate. These observations suggest that biofilm thickness modulates the relative contribution of neutral and deterministic processes on community assembly, with selection dominating in all biofilms, but stochastic factors playing a greater role in thicker biofilms. Furthermore, biofilm communities exhibited high temporal stability, which increased with thickness. We propose that the small, active volume of thin biofilms is subject to greater competition compared to thicker biofilms, where the presence of less active basal layers increases the contribution of neutral processes in community assembly.

Filling two needs with one deed – new generation biofertilizers enriched with greenhouse gas reducing bacteria

Åsa Frostegård*, Asa Frostegard, Daniel Mania, Kjell R Jonassen, Kedir Woliy, Phillip B Pope, Vincent GH Eijsink, Magnus Ø Arntzen, Live H Hagen, Lars R Bakken
Norwegian University of Life Sciences, Norway

Anthropogenic nitrogen will ultimately return to the atmosphere as N_2 , N_2O or NO , in proportions controlled by the ecology and regulatory biology of the microbes involved. Denitrification is a major source of N_2O , and substantial reductions of the N_2O emissions may be achieved by deliberately reducing the N_2O/N_2 product ratio by increasing the abundance of N_2O -reducing organisms. Manipulations of the indigenous soil microflora seem, however, to be an unrealistic strategy. Instead, this could be feasible using a suitable vector for industrial-scale introduction of N_2O -reducing organisms to soil. We have identified two such vectors. One is rhizobial inocula which enhance nitrogen fixation by leguminous crops. Many rhizobia are also denitrifiers. We investigated a large number of *Bradyrhizobium* and *Ensifer* strains, symbionts of economically important crops including soybean and alfalfa. Several were truncated denitrifiers, emitting N_2O . Others expressed N_2O reductase (N_2OR) and showed strong preference for N_2O - over nitrate-reduction, and we revealed by inhibiting the bc1 complex that this is due to N_2OR competing successfully with periplasmic nitrate reductase for electrons. The other option is to enrich biogas digestates, derived from biogas production, with N_2O reducing bacteria. This was done in anaerobically digested sewage sludge by anoxic incubation with N_2O . Metagenomic and metaproteomic analyses demonstrated dominance of the denitrifier *Dechloromonas denitrificans*, equipped with N_2OR type II. Soil amended with the N_2O -treated digestate emitted significantly less N_2O than the controls. This first proof of concept shows that the growing industry of anaerobic digestion provides a platform for low-cost reduction of N_2O emissions from soils.

Spores galore: bacterial survival revisited

Pilar Junier^{*1}, Thomas Junier², Christophe Paul¹, Sevasti Filippidou¹, Mathilda Hayoz¹, Anael Lehmann³, Patrick Chain⁴, Daniel Ariztegui⁵, Torsten Vennemann³,

Patrick Viollier⁵

¹University of Neuchâtel, Switzerland, ²Swiss Institute of Bioinformatics, Switzerland, ³University of Lausanne, Switzerland, ⁴Los Alamos National Laboratory, United States, ⁵University of Geneva, Switzerland

In most habitats fluctuating environmental conditions create periods of compromised survival for active organisms. In response, various strategies have evolved, including the formation of durable resting cells, often called spores. Up to now, spores have been described in a small number of bacterial clades. However, given the diversity of microbial ecosystems, there is a significant chance that other pathways based on different genes and generating uncharacterized spore-like cells remain to be discovered. We used a functional, phylogenetically unbiased approach to investigate the diversity of resting cells in the environment. This *environmental sporobiota* was defined based on the ability of these cellular structures to withstand a harsh DNA extraction method. The sporobiota community analysis detected three highly enriched phyla: Firmicutes, Actinobacteria and Proteobacteria, all known for spore production. However, detailed analysis unearthed many genera hitherto unknown to sporulate. The comparison of this fraction to the total bacterial community reveals the sporobiota to be a unique community and that production of a durable cell structure is a widespread environmental adaptation. Finally, we identify new taxa beyond known spore-formers with unique ecological properties and genetic diversity. Understanding dormancy is important for environmental management because these dormant populations might constitute a seed bank from which new communities might emerge after perturbation. Microorganisms are at the base of the functioning of the biosphere, the sporobiota is a unique component that cannot be ignored if we want to improve our ability to predict the response of the biosphere to environmental change.

Interplay of hospital microbiome and resistome

Kaisa Koskinen*, Stefanie Duller, Maximilian Mora, Manuela-Raluca Pausan, Christoph Högenauer, Robert Krause, Christine Moissl-Eichinger
Medical University of Graz, Austria

Hospital-acquired infections are a serious problem worldwide. The risk is not only related to invasive procedures or inadequate hygiene, but infection can also be transferred from patient to patient, via personnel, surfaces or equipment. Resistant (pathogenic) bacteria are frequently found in hospital surfaces, despite strict disinfection procedures.

In this project we explored the microbiome of a patient and patients' immediate surroundings at intensive care unit to identify the microbial communities and microbial transfer. We also compared the microbiomes and resistomes of areas with different cleaning and disinfection procedures, and studied if purposely increased environmental biodiversity in hospital can decrease the abundance of harmful microbial features, and hinder development and transfer of resistance elements.

We correlate the resistome information (epicPCR, plasmidome) and 16S rRNA gene-based microbial community composition with degree of confinement, cleaning and disinfection procedures and microbial diversity. Patient clinical parameters we associate with microbial community profile and diversity.

Human associated bacteria dominated the hospital indoor environment. Microbial communities in particular environments and patient body sites were highly specific, although several pathogenic bacteria including *Staphylococcus*, *Enterococcus*, *Pseudomonas*, *Klebsiella*, and *Acinetobacter* were detected both in patient and environment. Patient microbiome was more constant compared to environment, even when most of the microbial diversity was lost in specific body sites during the study. The microbial community in environment fluctuated reflecting the effect of disinfection and usage of sampled space, and compared to patient, carried more microbial diversity and features related to pathogenicity and resistance including mobile elements, biofilm formation and stress tolerance.

Comprehensive 16S/18S rRNA databases for microbial ecosystems in environmental biotechnology

Per H. Nielsen*, Vibeke Rudkjøbing, Jannie Kristensen, Marta Nierychlo, Francesca Petriglieri, Simon Knutson, Erika Yashiro, Søren Karst, Mads Albertsen, Per Halkjær Nielsen
Aalborg University, Denmark

Small subunit (SSU) ribosomal RNA genes have been used to study microbial diversity and evolution for the last 30 years. Databases containing full-length SSU rRNA reference sequences remain fundamental for many core analyses in microbial ecology, such as community profiling using 16S/18S rRNA amplicon sequencing and microscopy studies based on fluorescence *in situ* hybridization. The quality of the data produced relies heavily on the reference databases used, and we know that the current databases are underpopulated, ecosystem skewed, and subject to primer bias. We have developed a method to obtain millions of high quality, full-length SSU rRNA sequences without primer bias. The method combines reverse transcription of polyadenylated SSU rRNA molecules with Illumina based synthetic long-read sequencing and can be used to create comprehensive 16S/18S rRNA reference databases for any ecosystem. Here we demonstrate the power of the method using numerous samples from wastewater treatment plants in more than 30 countries, producing a “World catalogue of microbes in wastewater treatment systems”. More than 500,000 full-length 16S rRNA sequences reveal hitherto unknown bacterial and archaeal diversity, allow reevaluation of primers for amplicon sequencing, and design of improved FISH probes. This will greatly improve microbial community analyses and increase the resolution of *in-situ* single-cell physiology studies. Furthermore, it will be an invaluable reference resource for future studies of structure and function of microbial communities in environmental biotechnology.

Optimal performance prediction from coalesced acidophilic communities

Tomasa Sbaffi*¹, Pawel Sierocinski¹, Chris G. Bryan², Angus Buckling¹

¹*University of Exeter, United Kingdom*, ²*Bureau de Recherches Géologiques et Minières (BRGM), France*

Community coalescence (mixing of communities) is a process widely occurring both in nature and in industrial processes reliant on microbes. One example with high significance to the industry where coalescing communities is widely adapted is bio-hydrometallurgy. Typically during the process, communities from various environments are mixed in order to obtain an optimal inoculum for the bio-leaching of metals from minerals. The method is commonplace, however, its changes in community function and structure are poorly studied and understood.

Recent studies suggest that coalesced communities can behave as cohesive modules due to the coevolution of community members. This leads to one community dominating a coalesced community both in terms of the structure and function of the coalesced assembly. We tested if this applies to acidophilic communities from mine waste characterized by different geochemical properties. We mixed the communities in groups of two, three and four and fed them with pyrite. To assess the community function we used Fe oxidation, H⁺ production and cell abundance as proxies. To test the structure, we sequenced the communities at start and endpoint.

Our results show, consistently with previous research, that the mixtures containing the best individual community were the best functioning and the mix of all the communities was the best working treatment. Furthermore, the original community composition may help predicting the efficiency of the coalesced community. The findings obtained are relevant to the understanding of the ecological dynamics of microbial acidophilic communities as well as showing a simple method for predicting communities' properties for bio-leaching.

Link between prokaryotic N₂O production and fungal carbon degradation in soil

Christoph Keuschnig*, Catherine Larose
Ecole Centrale de Lyon - University of Lyon, France

Previous experiments on N₂O producing fungal cultures showed that N₂O production kinetics is lower in fungi than in bacteria by a factor of at least 10³. However, soil-based studies implicate fungi in a dominant role during N₂O emissions even though we cannot use enzyme kinetics to explain the quantity of N₂O found in fungi. We hypothesized that nitrogen cycling prokaryotic communities in soil depend on the presence of fungi. Three types of organic matter (paper pulp, sewage sludge and green compost) were added to the same agricultural soil in microcosms and soil was sampled at eight time points over 30 days of incubation. Fungicides were added as controls to inhibit fungal metabolic activity. Metatranscriptomes of total RNA (prokaryotic and eukaryotic transcripts) and poly-A-tail isolated mRNA (eukaryotic transcripts) and metagenomes were sequenced by NGS and analyzed. N₂O, CH₄ and CO₂ were also quantified in the microcosm headspace. N₂O production depended on the presence of fungi, whereas CH₄ was produced independent of fungal inhibition. Organic matter degradation signatures were primarily found in eukaryotic derived transcripts, whereas nitrogen-cycling related transcripts were found to be of prokaryotic origin. Quantification of marker genes by qPCR supported our genomic-based data. This is the first study of greenhouse gas emissions from soil co-dependending on fungal and bacterial communities. A link between the prokaryotic nitrogen cycling community and the carbon-degrading fungal community in soil was demonstrated. This link describes the functional relationships between fungal and bacterial communities during organic matter degradation and co-occurring greenhouse gas production.

Fungal-bacterial interactions in the decomposer food web in forest soil: it's all about recycling!

Rubén López-Mondéjar*¹, Vendula Brabcová², Martina Štursová², Tomáš Větrovský², Petr Baldrian²

¹*Institute of Microbiology of the Czech Academy of Sciences, Czech Republic*, ²*Institute of Microbiology of the CAS, Czech Republic*

Microorganisms are the main drivers of the carbon flow in forest soils, playing critical roles in the transformation of organic matter. Although both fungi and bacteria are involved in this process, the contribution of individual microbial taxa and the importance of fungal-bacterial interactions in decomposer food webs are poorly understood. Here we studied the roles of fungi and bacteria and their interactions in the decomposition of dead biomass of various origin (plant, fungal and bacterial) in a temperate forest soil using stable isotope probing (SIP) coupled with amplicon sequencing and metagenomics. We demonstrate that although both microbial groups are involved in the assimilation and mineralization of complex carbon

sources in soil, decomposer fungi are better suited to utilize plant biomass with low N content while the ability to utilize fungal and bacterial biomass, which is more N-rich, is more frequent among bacteria. In addition, both fungi and bacteria encode a diverse, distinct and complementary pool of carbohydrate-active enzymes specialized in the degradation of dead biomass of various origin. Our results demonstrate that food webs are networks with a high level of recycling of the microbial biomass pool rather than hierarchical structures with unidirectional flow of carbon as previously assumed. These findings reveal not only the complex structure of the soil food web and the genes involved in the decomposition process, but also point at the importance of the fungal-bacterial interactions in the food web assembly and in the nutrient cycling in forest soil.

It is no thoroughfare: Fungal highways enrich and allow selective bacteria with particular antibiotic resistance genes to be spatially dispersed

Rashid Nazir^{*1}, Du Shua², Ju-Pei Shen², Hangwei Hu³, Ji-Zheng He³

¹COMSATS Institute of Information Technology, Pakistan, ²State Key Laboratory of Urban and Regional Ecology, Research Centre for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, 100085, China, ³Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Parkville, Victoria, 3010, Australia, Australia

During the recent times, environmental antibiotic resistance genes (ARGs) and their potential transfer to other bacterial hosts of pathogenic importance are of serious concern. However, the dissemination strategies of such ARGs are largely unknown. As fungal highways have earlier been reported to transfer specific bacteria and even chemical substances, we hypothesized that saprotrophic soil fungi could differentially enrich the antibiotic resistant bacteria (ARBs) and subsequently contribute in spatial distribution of selective ARGs. Wafergen qPCR analysis of 295 different ARGs was conducted for manure treated pre-sterilized soil incubated or not with selected bacterial-fungal consortia. The qPCR assay detected unique ARGs specifically found in the mycosphere of ascomycetous and basidiomycetous fungi. Both fungi exerted potentially different selection pressures on ARBs, resulting in different patterns of ARGs dissemination (to distant places) along their respective growing fungal highways. The relative abundance of mobile genetic elements (MGEs) was significantly decreased along fungal highways compared to the respective inoculation points. Moreover, the decrease in MGEs and ARGs (along fungal highways) was more prominent over time which depicts the continuous selection pressure of growing fungi on ARBs for enrichment of particular ARGs in mycosphere. Such data also indicate the potential role of saprotrophic soil fungi to facilitate horizontal gene transfer within mycospheric environmental settings. Our study, therefore, advocates to emphasize the future investigations for such (bacteria-fungal) interactive microbial consortia for potential (spatial) dissemination of resistance determinants which could ultimately increase the ARGs exposure risk to human beings.

The composition and functional potential of *Penicillium* hypha-associated microbiomes in soil as revealed using a novel baiting approach

Mette Haubjerg Nicolaisen^{*1}, Xiuli Hao¹, Behnoushsadat Ghodsalavi², Yong-Guan Zhu³, Stefan Olsson⁴, Ole Nybroe¹

¹University of Copenhagen, Denmark, ²Aphea.Bio NV, Belgium, ³Institute of Urban Environment, Chinese Academy of Sciences, China, ⁴Fujian Agriculture and Forestry University, China

Colonization of fungal hyphae by bacteria are widespread in nature. However, the main drivers for assembly of hypha-associated bacterial communities, and their functional traits in soil systems, remain elusive. We therefore developed a novel baiting type microcosm to study bacterial colonization of hyphae in five natural soils with different physicochemical properties, and used two *Penicillium* species with different phosphorus solubilizing capacities as bait. This approach enabled us to investigate the impact of soil type as well as fungal phylogeny and functional potential on the hypha-associated bacterial community.

Amplicon sequencing of 16S rRNA genes showed that bacterial communities associated with *Penicillium* hyphae differed significantly from soil communities showing decreased diversity and less variation in taxonomic structure. Hypha-associated communities had an increased abundance of specific bacterial phyla and discriminative taxa. Furthermore, specific OTUs were enriched in hypha-associated communities of individual *Penicillium* species. At the overall community level, soil type exhibited a significant impact on bacterial communities associated with hyphae, whereas the effect of fungal phylogeny was not significant.

Functional potential, assessed by quantitative PCR analysis, revealed increased abundance of genes involved in inorganic phosphorus cycling in several hypha-associated communities; in agreement, an increased proportion of potential inorganic phosphorus solubilizing bacteria associated with the fungal hyphae. Further, the analysis showed enrichment of genes involved in phosphonate metabolism consistent with predictions of functional profiles made from taxonomic community composition. Taken together, we established that the *Penicillium* hyphae represent a unique niche where soil type and fungal species together orchestrate microbiome assemblage and functional potential.

Narnaviruses: novel players in fungal-bacterial symbioses

Laila P. Partida-Martínez^{*1}, Astrid N. Espino-Vázquez¹, José R. Bermúdez-Barrientos¹, José F. Cabrera-Rangel¹, Stephen J. Mondo², Teresa E. Pawlowska³, Cei Abreu-Goodger¹

¹Centro de Investigación y de Estudios Avanzados del I.P.N., Mexico, ²Lawrence Berkeley National Laboratory, United States, ³Cornell University, United States

Rhizopus microsporus is a mucoromycotan fungus of importance in ecology, agriculture, food production and public health. The study of *R. microsporus* has

acquired high interest with the discovery of an intracellular resident, *Burkholderia rhizoxinica*. This vertically-transmitted Gram-negative bacterium is responsible for the production of toxins that are crucial for the pathogenicity of *Rhizopus*. Additionally, the endobacteria are essential for the asexual reproduction of their host and also positively affect its sexual cycle, being necessary for abundant zygosporangium production. After more than a decade since the discovery of this unique bacterial-fungal symbiosis, we now identified a novel participant: the *Narnavirus*. Fungal viruses of the genus *Narnavirus* reside in the fungal cytosol and are characterized by a single positive strand of RNA that encodes a RNA-dependent RNA polymerase. Analyzing transcriptomic data of *R. microsporus*, we discovered *Narnavirus* sequences, which we later confirmed by RT-PCR. We then quantified by qPCR two *Narnavirus* strains, RmNV-20S and RmNV-23S, during fungal development and found the highest load at the stationary phase. Our experiments also revealed that viral infection is independent of the presence of endobacteria and is maintained through both asexual and sexual reproduction of the fungus. Currently, we are investigating the prevalence of these novel viral symbionts in members of *Rhizopus*, as well as their contribution to the biology of *R. microsporus* and its impact on fungal-bacterial symbioses.

Do bacteria and fungi have a fragrant language all their own?

Ruth Schmidt^{*1}, Paolina Garbeva²

¹INRS - Institut Armand Frappier, Canada, ²Netherlands Institute of Ecology (NIOO-KNAW), Netherlands

Communication among organisms is essential for the functioning of any ecosystem. It is recognized that in the ecosystem soil bacteria and fungi communicate with each other over long distances via the exchange of volatile organic compounds (VOCs). However, the molecular responses by bacteria to fungal VOCs remain unknown. Here we perform transcriptomics and proteomics analyses of the beneficial bacterium *Serratia plymuthica* PRI-2C exposed to VOCs emitted by the fungal plant pathogen *Fusarium culmorum*. We find that the bacterium responds to fungal VOCs with changes in gene and protein expression related to motility, signal transduction, energy metabolism, cell envelope biogenesis, and secondary metabolite production. Metabolomic analysis of the bacterium exposed to the fungal VOCs, gene cluster comparison, and heterologous co-expression of a terpene synthase and a methyltransferase revealed the production of the unusual terpene *sodorifen* in response to fungal VOCs. Investigating its ecological role showed that *sodorifen* is essential in the protection of maize when being infected with *F. culmorum*. These results suggest that terpenes are the *lingua franca* in the long-distance communication between fungi and bacteria, driving the inter-organismal communication between microbes and plants.

The foe of modern kitchens - synergistic interactions facilitate establishment of black yeast *Exophiala dermatitidis* in dishwasher biofilm communities

Jerneja Zupančič^{*1}, Prem Krishnan Raghupathi², Mette Burmølle², Søren Johannes Sørensen², Nina Gunde-Cimerman¹

¹University of Ljubljana, Biotechnical faculty, Department of Biology, Slovenia, ²University of Copenhagen, Department of Biology, Section of Microbiology, Molecular Microbial Ecology Group, Denmark

Extreme habitats are not only limited to natural environments, but also exist in manmade systems, for instance, household appliances such as dishwashers. Research on dishwasher microbiome started in 2011 with the discovery that black yeast, especially opportunistic *Exophiala dermatitidis*, colonizes rubber seals of domestic dishwashers. This global phenomenon attracted even more attention with the isolation of other fungal human opportunistic pathogens from dishwashers. Due to the emphasis on fungi, the bacterial community of dishwasher remained initially unexplored. To address this issue, bacterial and fungal diversity in biofilms isolated from rubber seals of 24 different household dishwashers was investigated using next-generation sequencing. Microbiome resulted in bacterial genera such as *Pseudomonas*, *Escherichia*, and *Acinetobacter*, and fungal genera such as *Candida*, *Cryptococcus*, and *Rhodotorula*, to be represented in most samples and all aforementioned genera are known to include opportunistic pathogens. This study also showed how specific abiotic conditions of the dishwashers impact the abundance of microbial groups and the interkingdom and intrakingdom interactions in these biofilms. Actual isolation of cultivable bacterial and fungal strains from a defined area of one square centimetre of rubber seal and further screening tests showed, which bacterial four-species consortia gain in biomass by incorporating *E. dermatitidis* in the biofilm and thus contribute to its promotion and abundance. The significance of our research is in identifying the microbial composition of biofilms formed in a broadly used household appliance, in describing how diverse abiotic conditions affect the composition and a novel phenomenon of cross kingdom synergy in dishwasher biofilms.

Disease-induced assemblage of a plant-beneficial bacterial consortium

Roeland Berendsen*, Gilles Vismans, Yang Song, Corne Pieterse, Peter Bakker
Utrecht University, Netherlands

Disease suppressive soils typically develop after a disease outbreak due to the subsequent assembly of protective microbiota in the rhizosphere. The role of the plant immune system in the assemblage of a protective rhizosphere microbiome is largely unknown. In this study, we demonstrate that *Arabidopsis thaliana* specifically promotes three bacterial species in the rhizosphere upon foliar defense activation by the downy mildew pathogen *Hyaloperonospora arabidopsidis*. The promoted bacteria were isolated and found to interact synergistically in biofilm formation *in vitro*. Although separately these bacteria did not affect the plant significantly, together they induced systemic resistance against downy mildew and promoted growth of the plant. Moreover, we show that the soil-mediated legacy of a primary population of downy mildew infected plants confers enhanced protection against this pathogen in a second population of plants growing in the same soil. Together our results indicate that plants can adjust their root microbiome upon pathogen infection and specifically recruit a group of disease resistance-inducing and growth-promoting beneficial microbes, therewith potentially maximizing the chance of survival of their offspring that will grow in the same soil.

Type VI secretion in plant-beneficial *Pseudomonas protegens* contributes to gut microbiome invasion and pathogenicity towards a plant pest insect

Christoph Keel*¹, Jordan Vacheron¹, Maria Péchy-Tarr¹, Silvia Brochet¹, Marina Stojiljkovic¹, Monika Maurhofer²

¹University of Lausanne, Switzerland, ²ETH Zurich, Switzerland

Bacteria of the *Pseudomonas protegens* group are well known for their plant-beneficial activities, which include pathogen suppression, plant growth promotion and plant defense induction. In addition, they exhibit insecticidal activities towards herbivorous larvae of Lepidopteran and Dipteran pest insects. Following ingestion by the larvae, the insecticidal pseudomonads colonize the gut, breach the intestinal barrier, invade the hemocoel and ultimately kill the insects. Various antimicrobial metabolites and toxins contribute to the pathogen and pest control abilities of *P. protegens*. The bacterium efficiently colonizes different ecological niches, notably plants and insects that are densely populated by competing bacteria. The molecular basis of niche competitiveness of *P. protegens* remains largely unexplored. Here, we investigated the role of the unique type VI secretion system (T6SS) of *P. protegens* in interbacterial competition, insect gut colonization and pathogenicity. The T6SS is a contractile transmembrane apparatus of Gram negative bacteria that functions like a deadly syringe to inject effector proteins with toxic and lytic activities directly into neighbouring cells. The genome of model strain *P. protegens* CHA0 harbours a gene cluster encoding the T6SS core apparatus and two distinct gene clusters encoding VgrG spike, effector and cognate immunity proteins. We demonstrate that these clusters are required for pathogenicity towards larvae of the

cabbage pest *Pieris brassicae*. The core apparatus and one of the VgrG clusters contribute to colonization of the insect gut, gut microbiome invasion and competition with specific microbiome members. Our results highlight the importance of T6SS in niche competitiveness and insect pathogenicity of *P. protegens*.

Dirty weeds and the friends they keep: ACD6 and its effect on microbes and immunity in field-grown *A. thaliana*

Derek Lundberg*, Wangsheng Zhu, Sonja Kersten, Karin Poersch, Julian Regalado, Efthymia Symeonidi, Detlef Weigel

Max Planck Institute for Developmental Biology, Germany

Accelerated Cell Death 6 (ACD6) is a transmembrane protein widely conserved in *A. thaliana* that functions in a positive feedback loop with the defense hormone salicylic acid (SA). It is primarily expressed in leaves, and helps to control expression of several immune receptors critical in detecting microbe-associated molecular patterns (MAMPs). Wild plant populations maintain variation in this gene, which have drastic phenotypic consequences in the lab. In particular, some accessions including Est-1 have a 'hyperactive' allele that results in necrosis in older leaves, overall higher SA levels, stunted growth, and more robust immunity to common pathogens. Other accessions such as Col-0 have 'weak' alleles that do not impart these phenotypes. Over two seasons, at a field site in Germany, at a field site in Sweden, and in a greenhouse, we grew in wild soil genotypes originating from Est-1 and Col-0 parents that have sequence variation only at the ACD6 locus, allowing for a direct test of the influence of ACD6 alleles on microbiome assembly, the plant immune response, and plant fitness. I will present results from a dataset of over 300 mature plants, showing for each plant visual phenotypes, 16S and ITS amplicon sequencing, metagenome sequencing, plant transcriptome sequencing, and plant hormone measurements including SA. Accompanying experiments include MAMP-response tests and bacterial growth curves on both greenhouse and field-grown plants. Counterintuitively for an immune regulator, ACD6 and associated immune responses are reduced in field grown plants despite those plants facing higher microbial load and microbial diversity.

Laying cables; redressing sulphide oxidation in seagrass rhizospheres

Belinda Martin^{*1}, Jeremy Bougoure¹, Timothy Colmer¹, Megan Ryan¹, Natalie Joyce¹, Ylva Olsen¹, Will Bennett², Gary Kendrick¹

¹*The University of Western Australia, Australia*, ²*Griffith University, Australia*

Seagrasses thrive in anoxic sediments where sulphide can accumulate to phytotoxic levels. So how do seagrasses persist in this environment? Here, we propose that oxygen loss from actively growing root tips protects seagrasses from sulphide intrusion not only by abiotically oxidising sulphides in the rhizosphere of young growing roots, but also by manipulating the abundance of sulphate-reducing and

sulphide-oxidising bacteria. We used a novel multifaceted approach combining imaging techniques (confocal fluorescence *in situ* hybridisation, oxygen planar optodes and sulphide diffusive gradients in thin films) with microbial community profiling to build a complete picture of the micro-environment of growing roots of the seagrasses *Halophila ovalis* and *Zostera mucronata*. Oxygen loss was restricted to actively growing root tips, indicating that, on the meadow-scale, seagrasses will have limited ability to promote sulphide oxidation. On the micro-scale, however, oxygen leakage corresponded with decreased abundance of potential sulphate-reducing bacteria and decreased sulphide concentrations in the rhizosphere surrounding growing roots. Furthermore, roots leaking oxygen had a higher abundance of sulphide-oxidising cable bacteria within the root hair zone; this is the first report of these bacteria in seagrass rhizospheres. Thus, oxygen leakage can enhance both abiotic and bacterial sulphide oxidation and restrict bacterial sulphide production around young vulnerable roots, thereby helping seagrasses to colonise sulphide rich anoxic sediments.

Breeding for resistance to soil-borne pathogen impacts rhizosphere microbiome in common bean

Lucas William Mendes^{*1}, Jos M. Raaijmakers², Rodrigo Mendes³, Siu Mui Tsai¹

¹University of Sao Paulo, Brazil, ²Netherlands Institute of Ecology, Netherlands, ³Embrapa Meio Ambiente, Brazil

The rhizosphere microbiome plays key role in plant growth and health, providing a first line of defense against root infections by soil-borne pathogens. Here, we investigated the composition and metabolic potential of the rhizobacterial community of common bean (*Phaseolus vulgaris*) cultivars with variable levels of resistance to the root pathogen *Fusarium oxysporum* (*Fox*). For the different bean cultivars the rhizobacterial abundance was positively correlated with *Fox*-resistance. Pseudomonadaceae, Bacillaceae, Solibacteraceae and Cytophagaceae were more abundant in the rhizosphere of the *Fox*-resistant cultivar. Network analyses showed a complex and highly connected bacterial community in the *Fox*-resistant. Specific functional traits such as protein secretion systems and biosynthesis genes of antifungal phenazines and rhamnolipids were more abundant in the rhizobacterial community of the *Fox*-resistant. Metatranscriptome data revealed that community assembly in the rhizosphere follows niche-based mechanisms, presenting lower diversity and distinct community structure comparing to the bulk soil. In comparison with the susceptible plant, the *Fox*-resistant cultivar presented high expression of genes affiliated to the family Paenibacillaceae, a group known by its antifungal activity. The *Fox*-resistant cultivar also presented high expression of genes related to metabolism of nutrients and specific functional traits related to pathogen suppression, such as motility and chemotaxis, and phenazine and colicin V. Our findings suggest that breeding for *Fox*-resistance in common bean have co-selected for plant traits that support a higher abundance of specific beneficial bacterial families in the rhizosphere with functional traits that support a more complex rhizosphere microbiome and reinforce the first line of defense against the pathogen.

Synthetic community experiments show a role of Alpha diversity and key players in plant protection in *Arabidopsis thaliana*

Barbara Müller*, Miriam Bortfeld-Miller, Julia Vorholt
ETH Zurich, Switzerland

The phyllosphere of plants harbours an abundant and diverse bacterial microbiota. Most of these microorganisms are not pathogenic and play an important role in plant health, as they can promote plant growth through production of hormones and are involved in plant protection, by producing antibiotic compounds, competing for nutrients or by activating the plant's immune system. Though playing an important role for plant health, microbial interactions and host-microbe interactions are not well understood. To get insights into community members relevant for plant protection, synthetic communities (SynCom) were designed from a collection of 200 genome-sequenced native strains isolated from healthy *A. thaliana* plants and a previously performed screen for protection against the foliar pathogen *Pseudomonas syringae* DC3000 *luxCDABE* (*Pst*). It could be shown that distinct SynComs protect the plant against *Pst* to different extent and that the complex SynCom, composed of all 200 strains, is more protective than low complex SynComs, composed of only 15 strains. Substituting strains with phylogenetically closely related strains in lower complexity SynCom experiments suggest that not only Alpha diversity, but also presence of key strains play an important role in plant protection.

Getting to the root of the issue: circadian rhythms in the rhizosphere microbiome

Amy Newman*, Emma Picot, Sally Hilton, Isabelle Carre, Gary Bending
University of Warwick, United Kingdom

The circadian clock is a widespread intracellular mechanism that allows organisms to synchronise their internal biological processes with predictable daily variations in their external environment. The circadian rhythms it generates are well studied in plant systems, with many aspects of plant physiology, development and metabolism known to be influenced by the clock. However, very little is known about the possible presence and influence of circadian rhythms in the rhizosphere microbiome, in which plant-microbe interactions at the root-soil interface can have significant implications for plant health. This work sought to determine whether a dysfunctional plant circadian clock influences the rhizosphere microbiome, and whether the microbiome composition changes between the morning and evening. Two *Arabidopsis thaliana* genotypes with different mutations in the plant clock gene *LHY* (late elongated hypocotyl, part of the core feedback loop), were grown in UK agricultural soil. Root and rhizosphere sampling of DNA and RNA was conducted at two time points twelve hours apart, and amplicon sequencing used to characterise bacterial and fungal communities. Significant differences at the whole community level were found between wild type control plants and both the mutants, indicating that the functional plant circadian clock does influence its microbiome. Further, individual Operational Taxonomic Units with significantly different relative abundances between morning and evening were found in all three plant genotypes tested, and these were found to

be genotype-specific. Therefore, the rhizosphere microbiome appears to be more variable over relatively short timescales than previously anticipated.

Metabolic potential and activity in the continental deep subsurface at the Sanford Underground Research Facility, South Dakota, USA

Jan Amend^{*1}, Magdalena Osburn², Lily Momper³, Gregory Fournier³, Cara Magnabosco⁴, Annette Rowe⁵, Karla Abuyen¹, Jayme Feyhl-Buska¹

¹*University of Southern California, United States*, ²*Northwestern University, United States*, ³*Massachusetts Institute of Technology, United States*, ⁴*Flatiron Institute, United States*, ⁵*University of Cincinnati, United States*

The continental subsurface is home to the largest biosphere on Earth. Access to this environment, especially the deeper parts, is extremely difficult, but can be achieved through surface springs, boreholes, and mines—each with pros and cons. We have established a Deep Mine Microbial Observatory (DeMMO) down to ~1500 m at the Sanford Underground Research Facility (SURF) in South Dakota, USA. There, we are monitoring regularly for cations, anions, redox-sensitive species, dissolved organic carbon, water isotopes, gas chemistry, and microbial cell counts and community composition. Calculated reaction energetics demonstrate catabolic potentials from a wide range of inorganic redox processes. Energy yields are up to ~120 kJ/mol e⁻ transferred, which is similar to maximum yields evaluated for high-energy continental and marine hydrothermal systems. An electrochemical approach is providing robust evidence of *in situ* metabolic activity at -200 mV redox potential, suggesting site-endemic biofilm-forming microbes capable of mineral redox reactions. Heat signals measured by nanocalorimetry indicate relatively low rates of microbial metabolic activity in unamended fluids. These activities, however, can be experimentally enhanced by the addition of various sources of carbon, nutrients, and energy. Metagenome assembled genomes of Chloroflexi, a cosmopolitan and ancient phylum of Bacteria, suggest that a number of subsurface members of this phylum acquired the genes for sulfur and nitrogen reduction pathways early in their evolutionary histories.

Comparative metagenomics highlighted a pathway involved in the catabolism of phosphonates in multiple serpentinizing ecosystems

Eleonore Frouin^{*1}, Fabrice Armougom², Gaël Erauso²

¹*Aix Marseille Universite, France*, ²*Mediterranean Institute of Oceanography, France*

Serpentinizing hydrothermal systems result from in-depth waters circulation and interactions with mantle-rocks in oceanic ridges or terrestrial ophiolites, producing alkaline fluids enriched in hydrogen, methane and small organics which are assumed to feed their microbial inhabitants. However, the functioning of these extreme ecosystems remains mostly unknown. Here, we explored the links between serpentinization and associated microbial communities through comparative metagenomics of serpentinite-hosted ecosystems, basalt-hosted vents, and hot springs. Both taxonomic and functional profiles showed that the microbiomes of the two submarine serpentinite-hosted systems, the Prony and Lost-City Hydrothermal Fields were more distant than expected from previous 16S rRNA surveys. Specifically, the microbial biosphere of Prony was more similar to that of terrestrial serpentinizing sites, while Lost-City metagenomes were more similar to those from

oceanic basalt-hosted vents. This study confirmed the importance of hydrogen-related metabolisms in serpentinizing sites but also revealed a set of highly enriched genes in serpentinizing ecosystems, which are involved in phosphonate catabolism. We estimate that up to 44% of the microorganisms in these ecosystems possess a C-P lyase, the key enzyme of this metabolic pathway. Despite being an essential element for life, inorganic phosphate is a limiting nutrient in many ecosystems. However, phosphonates constitute a significant fraction of dissolved organic phosphorus that can be used by microorganisms and lead to methane production. Our findings showed for the first time the importance of phosphonate degradation in serpentinizing systems, which is probably critical for their functioning and might even be linked to biotic production of methane, independently of methanogenesis.

Microbial ecology and activity of Mid-Atlantic Ridge oceanic crust through single cell -omics

Jacqueline Goordial*, Tim D'Angelo, Beth Orcutt
Bigelow Laboratory for Ocean Sciences, United States

Subsurface environments are often characterized by low biomass microbial communities with long generation times, resulting in difficult or impossible to obtain DNA and RNA for downstream genomic analyses of function and activity. We investigated whether flow cytometry could be used to concentrate cells to overcome the limitations due to low biomass in subsurface oceanic crust and sediment samples. We also explored the use of bioorthogonal noncanonical amino acid tagging (BONCAT) methods to fluorescently tag translationally active microorganisms coupled to flow cytometric sorting to separate active microorganisms from the bulk community of cells. This approach is a step forward to better understanding subsurface microbial systems through molecular techniques, since common methodologies rely on interpreting results from bulk communities of cells regardless of physiological state, including dead, dormant or non-growing cells. Here, we will present amplicon, metagenomic, and single cell genomic data from cells sorted from subsurface sediments and oceanic crust from the ultramafic Atlantis Massif and the mafic North Pond environments on the western flank of the Mid-Atlantic Ridge.

Anaerobic oxidation of ethane by globally abundant consortia-forming archaea

Cedric Hahn*¹, Rafael Laso-Pérez¹, Francesca Vulcano², Ida H. Steen², Runar Stokke², Manuel Liebeke¹, Andreas Teske³, Rudolf Amann¹, Katrin Knittel¹, Gunter Wegener¹

¹Max-Planck Institute for Marine Microbiology, Germany, ²Department of Biological Sciences, KG Jebsen Centre for Deep Sea Research, University of Bergen, Norway, ³Department of Marine Sciences, University of North Carolina, United States

Seafloor structures such as cold seeps and hydrothermal vents release large amounts of gaseous hydrocarbons that are oxidized under oxic or anoxic conditions, often with sulfate as electron acceptor. Whereas the organisms responsible for the anaerobic oxidation of methane or butane are well studied, little is known about organisms that consume ethane. To identify the microorganisms responsible for the latter process, we set up enrichment cultures from hydrothermally heated sediments of the Guaymas Basin, Gulf of California. The thermophilic enrichments were strongly dominated by archaea from the euryarchaeotal clade GoM-ArcI. GoM-ArcI formed consortia with *Candidatus Desulfofervidus*, a deltaproteobacterial genus known so far as thermophilic partner in the anaerobic oxidation of methane and butane. Metagenomic, transcriptomic and metabolomic analyses revealed that GoM-ArcI contains and highly expresses a methyl-coenzyme M reductase (MCR) which is a remarkably divergent variant of the known methanotrophic and methanogenic MCRs. The detection of ethyl-CoM as metabolite in the enrichments suggests the activation of ethane by an analogous reaction to the first step in the anaerobic oxidation of methane. From metagenomic reads we reconstructed an 84.53% complete draft genome of GoM-ArcI. A CARD-FISH-based survey showed that GoM-ArcI archaea are globally abundant in ethane-rich seep sediments with cell numbers ranging from 10^6 to 10^8 cells cm^{-3} . Depending on temperature GoM-ArcI cells form consortia with either *Candidatus Desulfofervidus* in heated sediments or with yet unidentified bacteria in cold seep sediments. As globally important ethane degrader, GoM-ArcI is adding a puzzle piece to the understanding of subsurface hydrocarbon cycling.

Thermophilic endospores associated with migrated thermogenic hydrocarbons in deep Gulf of Mexico marine sediments

Casey Hubert^{*1}, Anirban Chakraborty¹, Emily Ellefson¹, Carmen Li¹, Daniel Gittins¹, Bernie B Bernard², James M Brooks²

¹University of Calgary, Canada, ²TDI Brooks International, United States

Dormant endospores of thermophilic bacteria (thermospores) can be detected in cold marine sediments following high-temperature incubation. Thermospores in the cold seabed may be explained by a dispersal history originating in deep biosphere oil reservoir habitats where upward migration of petroleum fluids at hydrocarbon seeps transports viable cells into the overlying ocean. We assessed this deep-to-shallow dispersal hypothesis through geochemical and microbiological analyses of 111 marine sediments from the deep water Eastern Gulf of Mexico. GC-MS and fluorescence confirmed the unambiguous presence of thermogenic hydrocarbons in 71 of these locations, indicating seepage from deeply sourced petroleum in the subsurface. Heating each sediment to 50°C followed by 16S rRNA gene sequencing revealed several thermospores with a cosmopolitan distribution throughout the study area, as well as thermospores that were more geographically restricted. Among the thermospores having a more limited distribution, 12 OTUs from eight different lineages were repeatedly detected in sediments containing thermogenic hydrocarbons. A subset of these were significantly correlated with hydrocarbons ($p < 0.05$) and most closely related to *Clostridiales* previously detected in oil reservoirs from around the world. This provides evidence of bacteria in the ocean

being dispersed out of oil reservoirs via hydrocarbon seepage, and suggests that specific thermospores may be used as model organisms for studying warm-to-cold transmigration in the marine environment.

Linking deep life and deep time with ecological modeling

Cara Magnabosco
Flatiron Institute, United States

Little attention has been paid to how microorganisms came to populate the deep subsurface, how diversity was generated and maintained, and the dynamics that led to the cellular distributions we observe. To investigate these questions, we compiled a large collection of cell count and microbial diversity data from the deep marine and continental subsurface. Using cell counts from the continental subsurface and global maps of heat flow, surface temperature, lithology, and groundwater recharge, we provide a global map for the distribution of microorganisms in the continental subsurface. When combined with previous cellular estimates for subseafloor sediment and a revised marine crustal estimate based on the cellular abundances observed in basalts, we estimate that the subsurface biosphere holds $\sim 11 \times 10^{29}$ cells, approximately 70% of the world's bacteria and archaea. Using 16S SSU RNA gene surveys from around the globe, we compare the microbial diversity of the subsurface to other biomes around the globe and find that that subsurface samples contain more species than human-associated samples but do not exhibit scaling relationships that are frequently reported in ecological studies. Combined, these observations allow us to introduce a new model for how diversity is generated and maintained in the subsurface. Using these observations and ideas from traditional ecological modeling such as Lotka-Volterra predator-prey equations for viral-host dynamics, we estimate important ecological terms such as the rate of replication and infection.

Quantifying aerobic microbial activity in deep-sea red clay using H_2^{18}O

William Orsi^{*1}, Scott D. Wankel², Ömer Coskun¹, Magritsch Tobias¹, Aurele Vuillemin¹, Emily Estes³, Justine Sauvage⁴, Arthur Spivack⁴, David Smith⁴, Robert Pockalny⁴, Richard Murray⁵, D'Hondt Steve⁴

¹LMU Munich, Germany, ²Woods Hole Oceanographic Institution, United States, ³University of Delaware, United States, ⁴University of Rhode Island, United States, ⁵Boston University, United States

Subseafloor sediment underlying oceanic gyres contain deeply buried aerobic microbial communities living at the energetic limit to life. Biogeochemical models suggest aerobic microbes persist to great depths with ultra-slow respiration rates limited by organic matter scarcity. The small amount of biomass available has restricted microbiological studies to upper sediment layers, limiting our knowledge of

microbial diversity and activity under these extreme conditions. Here, we performed a long term quantitative stable isotope probing (qSIP) experiment with H_2^{18}O to measure aerobic activity of individual microbial taxa in 2.8 – 3.5 million-year-old deep-sea red clay. ^{18}O is incorporated into DNA from H_2^{18}O during genome replication, and is a passive tracer of microbial activity and growth. After 7 month incubations using oxic subseafloor sediment from two sites, qSIP identified 24 actively growing operational taxonomic units (OTUs), which increased to 44 OTUs after 18 months with 40% of ^{18}O -labeled OTUs being detected at both sites. OTUs affiliated with Thaumarchaea, Woeseearchaea, and Chloroflexi were most abundant throughout the sediment column at both sites, and also the most heavily enriched with ^{18}O thus providing a quantitative activity estimate for dominant subsisting taxa. Specific respiration rates of ^{18}O -labeled OTUs ranged between 186 and 10^{-4} femtomoles $\text{O}_2 \text{ d}^{-1}$ with doubling times ranging from 31 to 550 d^{-1} , indicating a high variability in activity rates. These data provide the first experimental estimates quantifying aerobic subseafloor microbial activity, supporting prior calculations and models.